Original Research

LGR6-dependent conditional inactivation of E-cadherin and p53 leads to invasive skin and mammary carcinomas in mice

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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;Cdh1^F;Trp53^F* female mice containing either heterozygous or homozygous *Cdh1^F* 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 *Cdh1$^{F/WT}$;Trp53$^{F/F}$* versus homozygous *Cdh1$^{F/F}$;Trp53$^{F/F}$* mice (778 versus 754 days, *p*=0.5). One *Cdh1^F* 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%) *Cdh1^F* heterozygous and 3/12 (25%) *Cdh1^F* 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.

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].
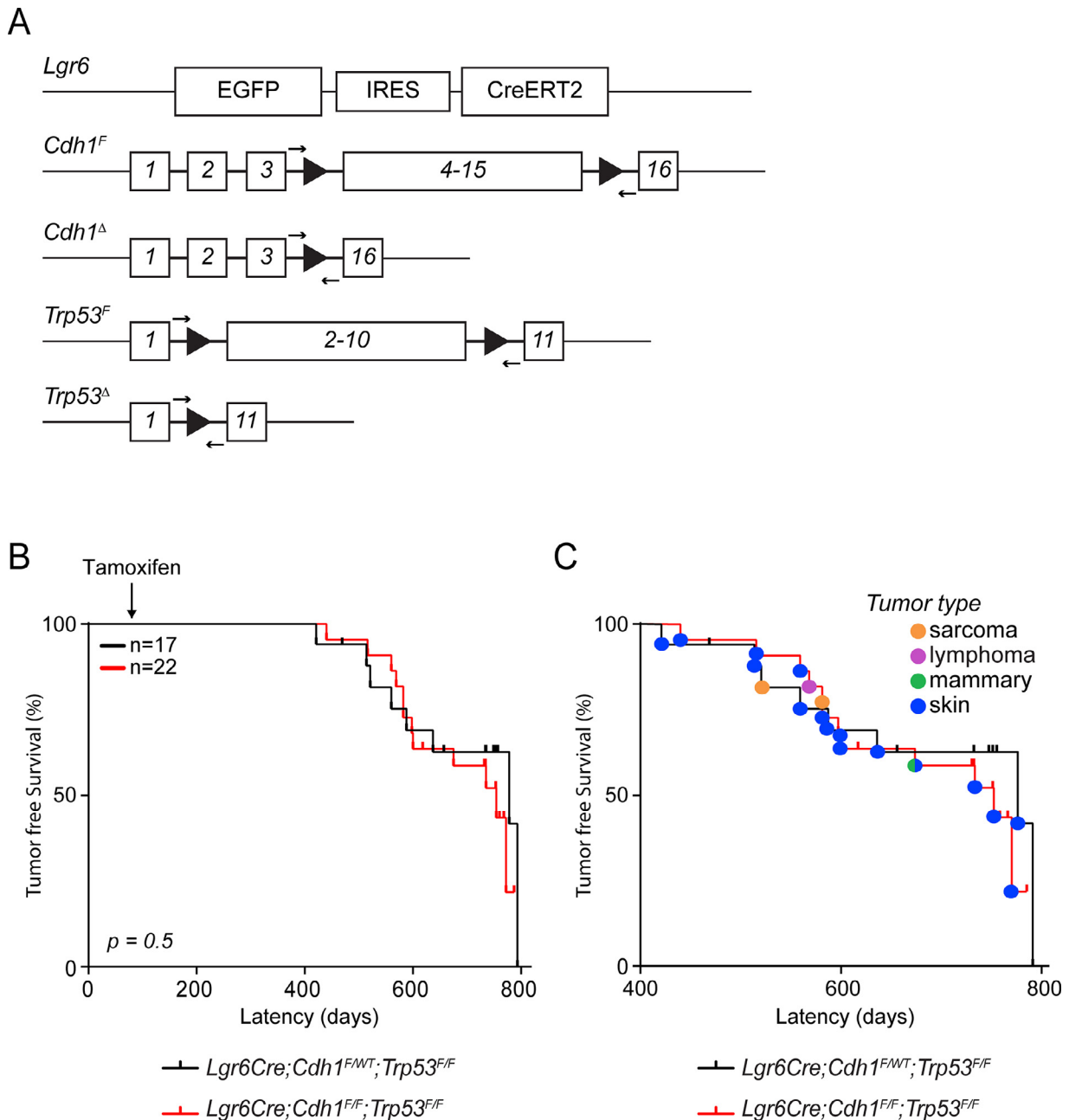
Fig. 1. Conditional deletion of *Cdh1* and *Trp53* drives tumor development in mice. A: Schematic model of tamoxifen induced Cre dependent deletion of the conditional *Cdh1^F* and *Trp53^F* alleles in Lgr6^{POS} cells. Eight to ten-week old mice were injected 3 times with tamoxifen to activate Cre in Lgr6^{POS} cells, resulting in deletion of the conditional *Cdh1^F* and *Trp53^F* alleles. Arrows indicate the positions of the genotyping primers. B: Kaplan-Meier tumor free survival curves of *Lgr6Cre;Cdh1$^{F/wt}$;Trp53$^{F/F}$* versus *Lgr6Cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* female mice ($p = 0.5$, log-rank test). Arrow indicates the time point of tamoxifen administration. C: Spectrum of tumors formed in *Lgr6Cre;Cdh1$^{F/wt}$;Trp53$^{F/F}$* and *Lgr6Cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* mice. Tumor types for each individual mouse are visualized in colored bullets. Only tumors with switched *Trp53^F* and/or *Cdh1^F* (Δ) alleles are shown in (B) and (C). For tumor details see Table S1.

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

Table 1

Inactivation of E-cadherin and p53 in Lgr6^{POS} cells induces carcinoma of the skin and mammary gland.

	Lgr6Cre;Cdh1$^{F/wt}$;Trp53$^{F/F}$	Lgr6Cre;Cdh1$^{F/F}$; Trp53$^{F/F}$	χ^2 p-value, df
Skin SCC			
Expansive	2/17 (12%)	1/22 (5%)	0.11, 1
Invasive	4/17 (24%)	9/22 (41%)	
Mammary gland			
Carcinoma	0/17 (0%)	1/22 (5%)	
Other			
Necrotizing dermatitis	1/17 (6%)	0/22 (0%)	
Histiocytic sarcoma	0/17 (0%)	1/22 (5%)	
Osteosarcoma	1/17 (6%)	0/22 (0%)	
Leukemic lymphoma	0/17 (0%)	1/22 (5%)	

SCC = Squamous cell carcinoma.

progenitor cells that contribute to alveolar expansion during pregnancy [17]. Moreover, Lgr6^{POS} epithelial progenitor cells were reported to underpin the development of luminal ER^{POS} mammary carcinomas in mice upon inactivating *Brca1* and *Trp53* mutations in these cells [17].

Given the reported retention of ER expression in *Lgr6-CreERT2;Brca1^F;Trp53^F* mice, we investigated the consequences of tamoxifen-induced inactivation of E-cadherin and p53 in Lgr6^{POS} 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-lobular mammary tumor in 1 mouse that progressed towards metastatic disease.

Materials and methods

Generation of Lgr6-EGFP-Ires-creERT2;Cdh1^F;Trp53^F female mice

Lgr6Cre;Cdh1^F;Trp53^F mice were generated by crossing heterozygous female *Lgr6-EGFP-Ires-creERT2* (Lgr6Cre) mice [20] with male *Cdh1$^{F/F}$;Trp53$^{F/F}$* mice [5]. The resulting heterozygous *Lgr6Cre;Cdh1$^{F/wt}$;Trp53$^{F/wt}$* offspring was backcrossed onto homozygous *Cdh1$^{F/F}$;Trp53$^{F/F}$* mice and intercrossed to produce female *Lgr6Cre;Cdh1$^{F/wt}$;Trp53$^{F/F}$* (n=17) and *Lgr6Cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* (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 mm^3 (mammary tumors), or 1,000 mm^3 (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 mm^3 was reached. All animal experiments were performed in accordance with local, national, and European guidelines under permit AVD115002015623 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, *Trp53^F*, *Trp53$^\Delta$*, *Cdh1^F*, and *Cdh1$^\Delta$* 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 36; 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 Lgr6^{POS} cells induces tumor formation

To study the oncogenic effect of tumor suppressor inactivation in Lgr6^{POS} progenitor cells, we crossed *Lgr6-EGFP-Ires-creERT2* (Lgr6Cre) mice [20] with conditional E-cadherin and p53 (*Cdh1^F;Trp53^F*) mice [5]. Heterozygous E-cadherin *Lgr6Cre;Cdh1$^{F/wt}$;Trp53$^{F/F}$* and homozygous E-cadherin *Lgr6Cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* mice (8-10 weeks old; n=39) were injected with tamoxifen to induce Cre recombinase-mediated inactivation of the conditional alleles in LGR6 expressing cells (Fig. 1A). Both heterozygous *Lgr6Cre;Cdh1$^{F/wt}$;Trp53$^{F/F}$* and homozygous *Lgr6Cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* 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;Cdh1$^{F/wt}$;Trp53$^{F/F}$* and 12 out of 22 (55%) *Lgr6Cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* mice up to a period of 800 days, of which most were skin carcinomas (Table 1). Homozygous deletion of *Cdh1^F* did not accelerate development of cancer in *Lgr6Cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* compared to heterozygous *Lgr6Cre;Cdh1$^{F/wt}$;Trp53$^{F/F}$* mice (p=0.5). The genetic status of *Cdh1* and *Trp53* was determined in all tumors that developed in the
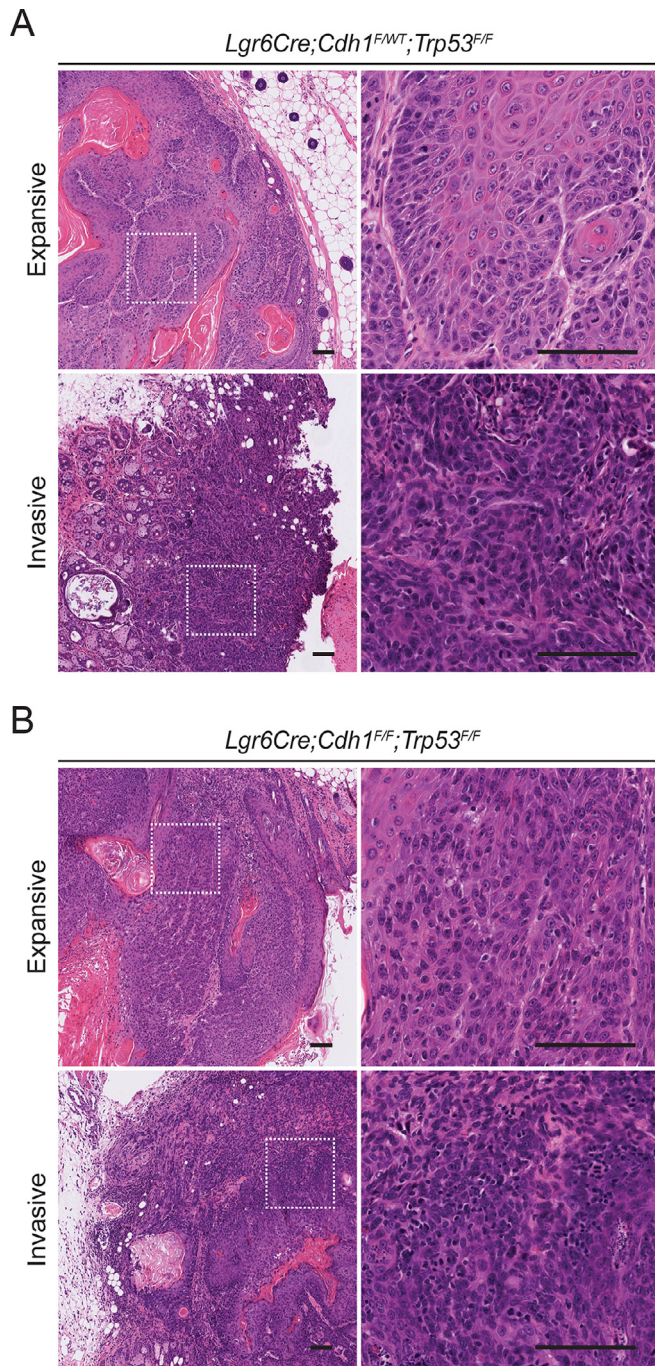
Fig. 2. Conditional inactivation of E-cadherin and p53 in Lgr6[POS] cells induces skin squamous cell carcinoma. A&B: H&E stained sections of skin squamous cell carcinomas (SCC) that developed in *Lgr6Cre;Cdh1[F/WT];Trp53[F/F]* (A) or *Lgr6Cre;Cdh1[F/F];Trp53[F/F]* (B) female mice with expansive and invasive phenotypes. Insets in the left panels depict the zoomed image in the right panels. Scale bars, 100 μm.

Fig. 3. E-cadherin and CK14 expression in SCCs of *Lgr6Cre;Cdh1[F/WT];Trp53[F/F]* and *Lgr6Cre;Cdh1[F/F];Trp53[F/F]* mice. A and B: Immunohistochemical analysis on SCC that developed in Lgr6Cre;Cdh1[F/wt];Trp53[F/F] (A) or *Lgr6Cre;Cdh1[F/F];Trp53[F/F]* 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.

Lgr6Cre;Cdh1[F/wt];Trp53[F/F] and *Lgr6Cre;Cdh1[F/F];Trp53[F/F]* mice (Table S1). Homozygous loss of the conditional *Trp53* alleles was detected in all skin and mammary tumors, whereas the conditional *Cdh1* was retained in some tumors that developed in both *Lgr6Cre;Cdh1[F/wt];Trp53[F/F]* and *Lgr6Cre;Cdh1[F/F];Trp53[F/F]* mice. These findings suggest that, in contrast to
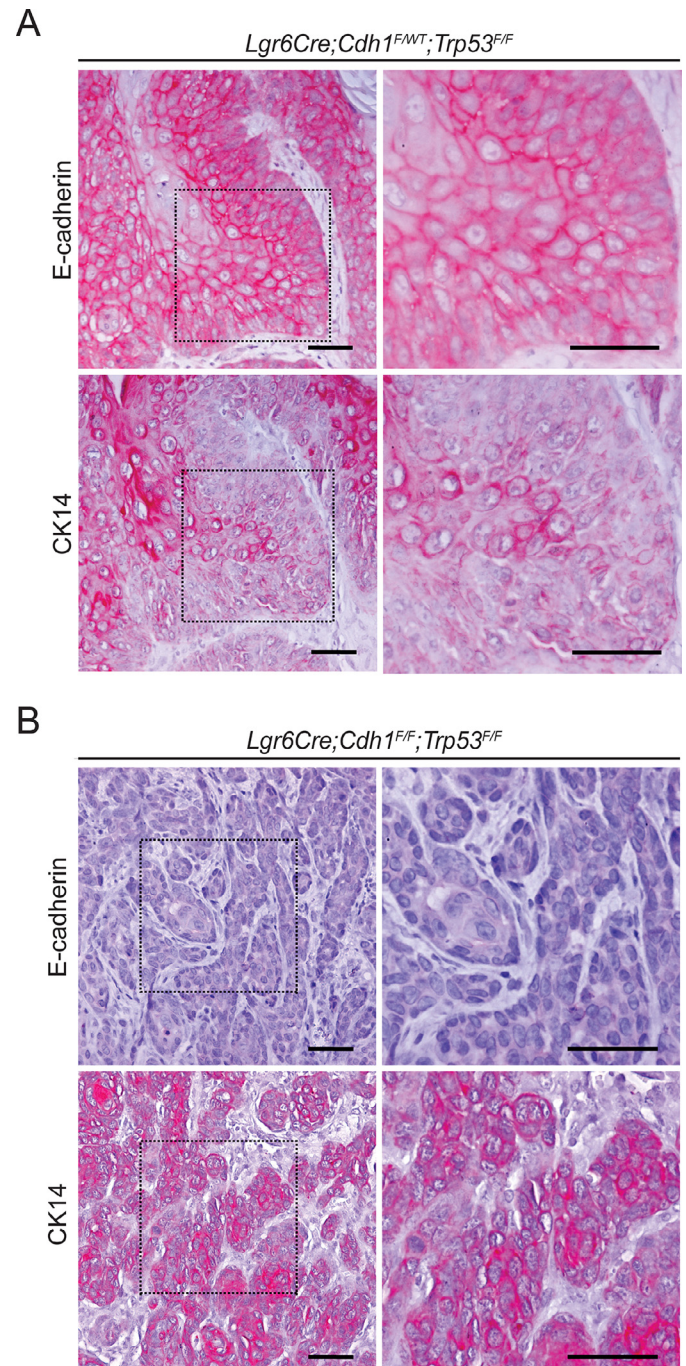
previous studies using K14cre [5,16], homozygous loss of E-cadherin does not provide a selective advantage for Lgr6[POS] cancer stem cells in the skin (Table S1). We also observed the development of lymphomas in 4/22 (18%) *Lgr6Cre;Cdh1[F/F];Trp53[F/F]* mice, but only one lymphoma showed switching (deletion) of the conditional p53 alleles (Table 1 and S1). In contrast

to the *Lgr6Cre;Cdh1$^{F/wt}$;Trp53$^{F/F}$* heterozygous mice, one homozygous *Lgr6Cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* mouse developed a mammary tumor(1/22, 5%) (Table 1), suggesting that in this model, bi-allelic deletion of *Cdh1* may be detrimental to the induction of mammary tumor formation in Lgr6^{POS} cells. Altogether, our data show that concomitant loss of E-cadherin and p53 in Lgr6^{POS} cells in mice results in the modest formation of skin and an occasional mammary tumor.

Inactivation of E-cadherin and p53 in Lgr6 expressing cells induces skin squamous cell carcinoma

Inactivation of E-cadherin and p53 in Lgr6^{POS} cells induced skin tumor formation in 6/17 (35%) *Lgr6Cre;Cdh1$^{F/wt}$;Trp53$^{F/F}$* mice and 10/22 (45%) *Lgr6Cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* mice. Skin tumors were predominantly diagnosed as squamous cell carcinomas (SCC) with either expansive or invasive growth patterns (Table 1) and (Fig. 2). Although we observed more invasive carcinomas in the E-cadherin homozygous cohort, this difference was not statistically significant when comparing the development of expansive *versus* invasive carcinomas in *Lgr6Cre;Cdh1$^{F/wt}$;Trp53$^{F/F}$* and *Lgr6Cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* mice (Table 1, p=0.11). SCCs were mostly formed in head and neck regions or the left and right flanks with no differences in tumor sites between both mouse cohorts (Table S1). One *Lgr6Cre;Cdh1$^{F/wt}$;Trp53$^{F/F}$* mouse diagnosed with invasive SCC presented with lung metastasis (Fig. S1B). Additional IHC confirmed loss of E-cadherin protein expression in the tumors that developed in *Lgr6Cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* mice, in contrast to the tumors that developed in Cdh1 heterozygous female mice (Fig. 3A,B). Cytokeratin-14 (CK14) was heterogeneously expressed throughout all SCC samples (Fig. 3C,D). Since the conditional *Cdh1* and *Trp53* alleles were deleted specifically in Lgr6-EGFP-Ires-CreERT2 cells, we determined the presence of Lgr6^{POS} cells in the SCC samples using the surrogate GFP marker (see Fig. 1A). GFP expressing Lgr6^{POS} cells were detected in the non-neoplastic skin cells surrounding the tumor front, but not in the tumor cells (Fig S2 A,B), suggesting that Lgr6 expression does not contribute to tumor maintenance or progression.

Mammary gland carcinoma development in Lgr6Cre;Cdh1^{F};Trp53^{F} mice

Because Lgr6^{POS} progenitor cells in the mouse mammary gland have been advocated as a tumor initiating cell [17], we investigated the consequences of *Cdh1* and *Trp53* loss in the *Lgr6Cre;Cdh1^{F};Trp53^{F}* model. In contrast to the frequent formation of skin tumors, we observed incidental mammary carcinoma development in one *Lgr6Cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* female mouse (1/22, 5%) (Table 1). The mammary tumor was classified as mammary gland carcinoma with squamous metaplasia (Table 1 and Fig. 4A). Additionally to the mammary carcinoma, this mouse also developed a SCC that was localized proximal to the tumor bearing mammary gland, as well as a metastatic lesion in the lungs (Fig S1A and Table S1). Histomorphological analysis indicated that the metastatic cancer cells originated from the mammary carcinoma, as metastatic lesions contained nest-like structures with characteristic nuclear atypia similar to the mammary carcinoma (Fig. S1A, left and middle panels). In contrast, cells from the primary invasive skin tumor contained abundant cytoplasm and formed keratin pearls (Fig. S1A, right panels), a feature that was also observed in a lung metastasis originating from a primary SCC (Fig S1B). As expected, we did not detect plasma membrane-localized E-cadherin expression in the mammary tumor that developed in the *Lgr6Cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* female mouse. (Fig. 4B). Basal CK14 expression was diffuse while luminal CK8 and ERα were not expressed in the mammary carcinoma (Fig. 4C-E). In line with its expression pattern in the skin, we did not observe Lgr6^{POS} cells in the mammary tumors while we did find expression in the basal layer of healthy epithelium (Fig. S2 C, D).

In summary, these data indicate that homozygous deletion of *Cdh1* and *Trp53* 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 *Brca1* and *Trp53* using Lgr6-Cre leads to ER^{POS} mammary carcinomas with a tumor-free latency period of approximately 1 year [17]. Unfortunately, while one mammary carcinoma developed in the *Lgr6Cre;Cdh1^{F};Trp53^{F}* female mice, we mainly observed the development of squamous skin tumors. Moreover, and in contrast to the published *Lgr6Cre;Brca1^{F};Trp53^{F}* model [17], we observed an average tumor-free survival latency of 766 days. This was a surprising finding, given that both studies used the same Lgr6Cre mouse model and compound *Cdh1^{F};Trp53^{F}* mice that have a near identical genetic background as the *Brca1^{F};Trp53^{F}* model. Furthermore, our experimental induction of Cre in mice using tamoxifen was based on the published materials of the aforementioned study [17]. 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^{F};Trp53^{F}* 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 *Trp53* using Lgr6Cre predominantly resulted in the formation of invasive SCC in mice. Development of skin SCC in the *Lgr6cre;Cdh1^{F};Trp53^{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 latencies 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* alleles, 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 either 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
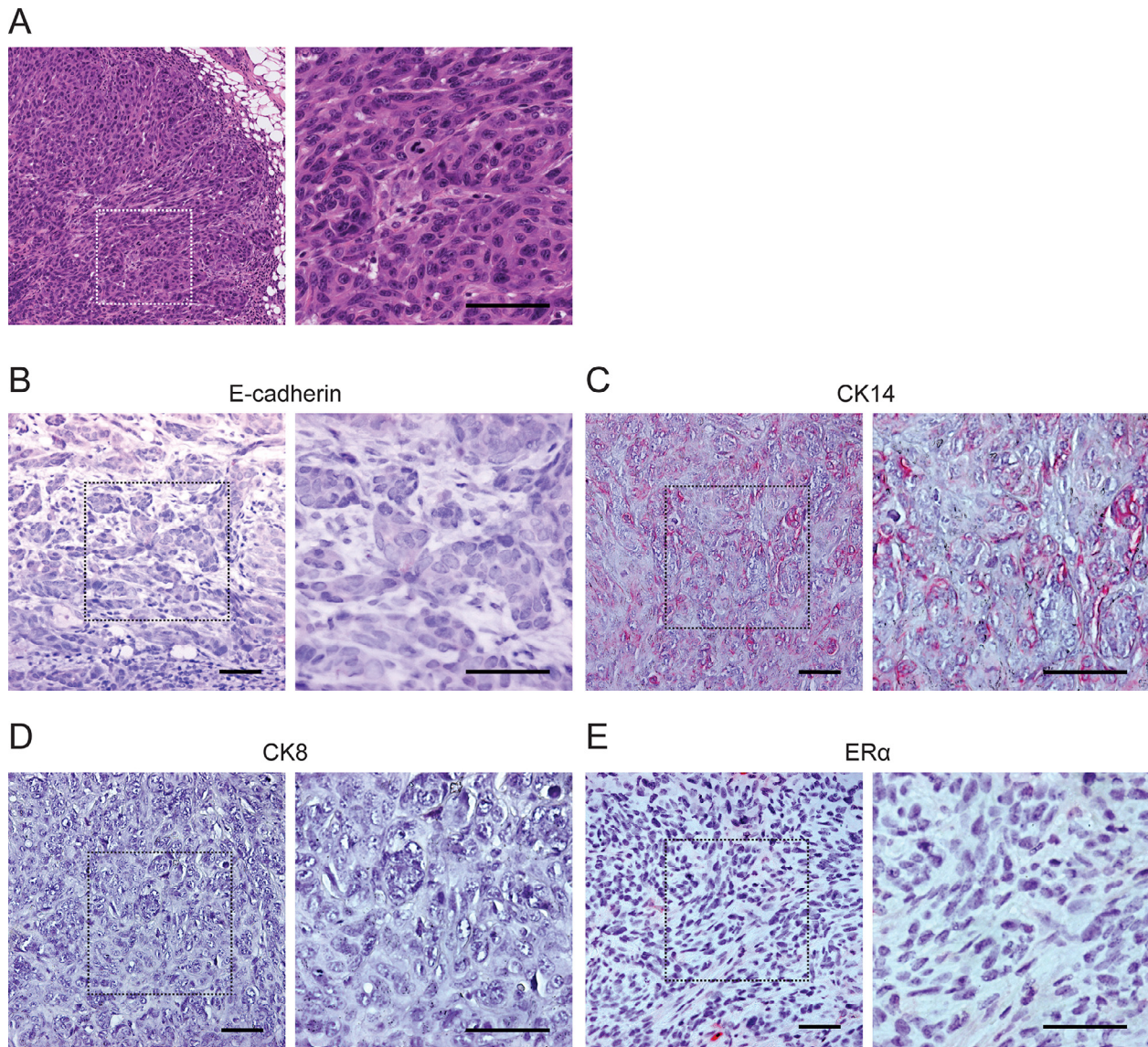
Fig. 4. Homozygous deletion of *Cdh1* and *Trp53* in Lgr6[POS] cells induces sporadic mammary carcinoma formation. A: H&E stained sections of an invasive mammary gland carcinoma that developed in a *Lgr6Cre;Cdh1^{F/F};Trp53^{F/F}* female mouse. Insets in the left panel depicts the zoomed image in the right panel. Scale bars, 100 μm. B-E: Immunohistochemical analysis of the mammary carcinoma shown in (A), analyzed for protein expression of E-cadherin (B), CK14 (C), CK8 (D) and ERα (E). Insets in the left panels depict the zoomed image in the right panels. Scale bars, 100 μm.

epidermis (IFE), the central isthmus and sebaceous gland, CK14[POS] 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;Cdh1^{F/wt};Trp53^{F/F}* and *Lgr6Cre;Cdh1^{F/F};Trp53^{F/F}* mice. Of note, Lgr6[POS] 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 Lgr6[POS] cells and as such hinders tumor formation. While deletion of E-cadherin and p53 in Lgr6[POS] 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 Lgr6[POS] 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 Lgr6[POS] 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.

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