The Mammary Progenitor Marker CD61/β3 Integrin Identifies Cancer Stem Cells in Mouse Models of Mammary Tumorigenesis

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Abstract

The cells of origin and mechanisms that underpin tumor heterogeneity in breast cancer are poorly understood. Here, we have examined three mouse models of mammary tumorigenesis (MMTV-wnt-1, MMTV-neu, and $p53^{+/-}$) for changes in their epithelial cell hierarchy during the preneoplastic and neoplastic stages of tumor progression. In preneoplastic tissue, only MMTV-wnt-1 mice showed a perturbation in their epithelial subpopulations. In addition to an expanded mammary stem cell pool, repopulating cells capable of yielding extensive mammary outgrowths *in vivo* were revealed in the committed luminal progenitor population. These findings indicate that wnt-1 activation induces the appearance of aberrant progenitor cells, and suggest that both mammary stem and progenitor cells can serve as the cellular targets of wnt-1–induced tumorigenesis. In tumors arising in MMTV-wnt-1 tumors, the luminal epithelial progenitor marker CD61/β3 integrin identified a cancer stem cell (CSC) population that was highly enriched for tumorigenic capability relative to the $CD61^-$ subset. CD61 expression also defined a CSC subset in 50% of $p53^{+/-}$–derived tumors. No CSCs, however, could be identified in the more homogeneous MMTV-neu/erbB2 model, suggesting an alternative model of tumorigenesis. Overall, our findings show the utility of the progenitor marker CD61 in the identification of CSCs that sustain specific mammary tumors. [Cancer Res 2008;68(19):7711–7]

Introduction

Mouse models of mammary tumorigenesis have been used extensively to investigate the genetic pathways that lead to mammary tumorigenesis (1). Delineation of the cell types within mammary tissue that are predisposed to tumorigenesis requires a detailed understanding of the cellular hierarchy. In the normal mouse mammary gland, mammary stem cells (MaSCs) with a phenotype of $CD29^{hi}CD49f^{hi}CD24^{mod/+}$ have been identified (2–4) and shown to regenerate a complete functional mammary gland at the single-cell level. This population, however, also comprises mature myoepithelial cells and presumptive basal progenitor cells. More recently, luminal progenitor cells have been defined based on expression of either CD61 (5) or CD133/prominin-1 (6), where the progenitor is $CD29^{lo}CD24^+CD61^+$ or $CD24^{hi}CD133^-$. More mature luminal cells, on the other hand, exhibit a phenotype of $CD29^{lo}CD24^+CD61^-$ or $CD24^{hi}CD133^+$. In addition to differentiated cells, the $CD29^{lo}CD24^+CD61^-$ subset contains a small proportion of progenitor cells (5).

Two predominant theories have been postulated to account for tumor heterogeneity and tumor propagation: the cancer stem cell (CSC) and clonal evolution models (7–9). A stochastic model may also apply in some cases, in response to microenvironmental factors. CSCs are defined as tumor cells that are capable of self-renewal and extensive proliferation, and possess at least some differentiative ability, thus sharing properties with normal tissue stem cells. These cells sustain tumorigenesis but are distinct from the cell of origin. CSCs were first prospectively isolated from leukemia (10), and there is accumulating evidence for their existence in diverse solid tumor types. In breast cancer, a small subset of $CD44^+CD24^{lo/-}$ cells prospectively isolated from human tumors was shown to be highly enriched for tumor-forming capacity (11), and ALDH-1 was also recently reported as a marker of human breast CSCs (12). Additionally, CSCs exhibiting a $CD24^+Thy\text{-}1^+$ phenotype have been prospectively isolated from mouse mammary tumors that develop in MMTV-wnt-1 transgenic mice (13).

To gain insight into the cell of origin and cellular mechanisms underlying tumor propagation, we have evaluated epithelial cellular subpopulations in preneoplastic and neoplastic mammary lesions in three mouse models (MMTV-wnt-1, MMTV-neu, and $p53^{+/-}$). Our findings revealed a substantial proportion of repopulating cells in the luminal progenitor subset isolated from MMTV-wnt-1 preneoplastic mammary glands. This suggests that wnt-1 signaling can confer stem-like properties on committed progenitor cells and/or that transformation of MaSCs is associated with some decrease in CD29 expression. In the case of established mammary tumors, we report that the luminal progenitor marker CD61/β3 integrin identifies a CSC population in MMTV-wnt-1 and $p53^{+/-}$ tumors that is markedly enriched for tumor-forming capacity. Our data provide further evidence that breast cancers can develop according to a CSC model of mammary tumorigenesis and highlight the importance of the progenitor marker CD61 in defining a cellular hierarchy within some tumors. Pertinently, the αv/β3 integrin complex has been implicated as a prognostic indicator in breast cancer and in regulating metastasis (14, 15).

Materials and Methods

Mice. The MMTV-wnt-1 (BALB/c), BALB/c-$p53^{+/-}$, and MMTV-neu (FVB/N) mice and their genotyping have been described previously (16–18). All animal experiments were conducted according to the Walter and Eliza Hall Institute (WEHI) Animal Ethics Committee guidelines. Mammary fat pad transplantation was carried out as described (2). Mice bearing tumors

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up to 1 cm in size were euthanized and the tumor was excised. For tumor development, mice were kept up to 4 mo. As cells were injected into both inguinal mammary glands, tumors were resected from anaesthetized mice to allow tumor development in the contralateral fat pad. All glands were processed by whole mounting and sectioning.

Preparation of mammary cell suspensions. The thoracic and inguinal mammary glands from wild-type or mutant mice were dissected from virgin female mice and mammary epithelial cell suspensions were prepared as described (2). For tumor cell suspensions, the following modifications were made: tumors were chopped with a razor blade and then sequentially digested with collagenase/hyaluronidase (300 units/mL and 100 units/mL, 50 min at 37°C), TEG (0.25% trypsin/1 mg/mL EGTA, 1 min at 37°C), and dispase (5 mg/mL, 5 min at 37°C).

Antibodies and cell sorting. Antibodies against mouse antigens were purchased from BD PharMingen, unless otherwise specified, and included CD24-PE, biotinylated CD31, CD45, and TER119; CD29-FITC (Chemicon); CD61-APC (Caltag); and streptavidin-APC-Cy7 and streptavidin-Alexa-594 (Molecular Probes). Antibody staining and cell sorting was as described (2). For labeling of intracellular epitopes, cells were fixed in chilled acetone for 1 min and permeabilized in 0.1% Tween/PBS on ice for 5 min before blocking. Antibodies were anti-cytokeratin 14 (Covance), anti-cytokeratin 18 (Progen Biotechnik), and anti-smooth muscle actin (SMA; Sigma).

Immunohistochemistry. Tumor fragments were fixed in 4% paraformaldehyde or 10% neutral-buffered formalin before paraffin embedding and sectioning. Primary antibodies included keratin 8 (K8; Fitzgerald), keratin 14 (K14; Covance), SMA (Sigma), and β-catenin (BD Biosciences) followed by secondary antibody (Vector) and final detection with avidin-biotin complex method reagent (Vector) and 3,3′-diaminobenzidine (DAKO).

Statistics. Analysis of the tumor-forming frequency was calculated using WEHI web interface[3] based on the limdil function in the statmod package.[4]

Results

Wnt-1 confers stem-like properties on luminal progenitor cells in preneoplastic mammary tissue. To identify potential "cells of origin" of tumors in the MMTV-wnt-1 (18), MMTV-neu/erbB2 (16), and $p53^{+/-}$ (17) models of mammary tumorigenesis, we evaluated preneoplastic tissue. Wnt-1 was first identified as a frequent proviral insertion site of MMTV, whereas erbB2/HER2 overexpression and p53 loss characterize a significant proportion of breast cancers. Single-cell suspensions prepared from mammary tissue were depleted of hematopoietic and endothelial cells using CD45, TER119, and CD31 (yielding the "Lin^-" population) and double sorted based on expression of CD29 (β1 integrin), CD24, and CD61 (β3 integrin). As previously noted (2), the $Lin^-CD29^{hi}CD24^+$ (referred to as $CD29^{hi}CD24^+$ from hereon) population was expanded in preneoplastic tissue from MMTV-wnt-1 mammary glands at 8 weeks of age compared with age-matched control glands (Fig. 1*A*), despite an age-related increase in the MaSC-enriched population. In functional assays of mammary fat pad repopulation (Table 1), this was associated with a 4-fold increase (4.34 ± 2.17 SE) in the absolute number of MaSCs in the $CD29^{hi}CD24^+$ population. In contrast, no significant increase in the number of MaSCs was observed in the $CD29^{hi}CD24^+$ population of $p53^{+/-}$ or MMTV-neu preneoplastic mammary tissue (1.25 ± 0.4 and 1.71 ± 1.05, respectively; Supplementary Table S1).

Notably, extensive mammary epithelial outgrowths were observed in transplants of luminal progenitor $CD29^{lo}CD24^+CD61^+$ cells from MMTV-wnt-1 mammary glands, occurring at a frequency of 1 in 464 cells relative to 1 in 111 for the MaSC-enriched $CD29^{hi}CD24^+$ population (Table 1). These outgrowths were severely hyperplastic (Fig. 1*B*) and comprised both luminal and myoepithelial cells based on immunostaining for K8 and SMA expression, respectively (Fig. 1*C*), showing bilineage potential. Interestingly, fluorescence-activated cell sorting (FACS) analysis revealed that $CD29^{lo}CD24^+CD61^+$ cells in MMTV-wnt-1 glands expressed high levels of cytokeratin 14 compared to those from wild-type glands (Supplementary Fig. S1). Outgrowths were also occasionally generated from the more mature $CD29^{lo}CD24^+CD61^-$ population, presumably emanating from progenitors in this subset (Table 1). There was no apparent change in CD61 expression in the $CD29^{lo}CD24^+$ luminal population between MMTV-wnt-1 preneoplastic tissue and aged-matched controls (23.4 ± 4% and 17.6 ± 6%, respectively). In addition, no obvious bias was observed in the expression of activated β-catenin (readout for wnt-1 activity) among the different subpopulations: there were 39.4%, 38.4%, and 32.6% β-catenin–positive (nuclear) cells in the $CD61^+$, $CD61^-$, and $CD29^{hi}$ subsets, respectively, based on immunostaining of freshly sorted cytospun cells. In contrast to MMTV-wnt-1 mice, neither $CD29^{lo}CD24^+CD61^+$ nor $CD29^{lo}CD24^+CD61^-$ cells from either $p53^{+/-}$, MMTV-neu, or wild-type mammary glands generated any outgrowths, compatible with data from multiple experiments (2).[5] The presence of repopulating cells in the luminal subsets of MMTV-wnt-1 mammary glands suggests that inappropriate wnt signaling has perturbed the mammary epithelial hierarchy. Further, the refinement of markers is required to determine the cells of origin in the MMTV-neu and $p53^{+/-}$ models.

Down-regulation of CD29 in the MaSC-enriched population of mammary tumors. We next examined changes in cellular subsets of established tumors. Unexpectedly, tumors from the three models studied shared a similar profile of CD29 and CD24 expression, with $CD24^+$ cells expressing more uniform levels of CD29 compared with the wider range observed in normal mammary epithelial cells (Fig. 2*A*). Thus, the transition from preneoplasia (Fig. 1*A*) to tumor formation (Fig. 2*A*) is characterized by loss of the $CD29^{bright}$ subset, compatible with the decrease in CD29 expression apparent in PyMT-induced tumors as they progress toward malignancy (19).[5] Furthermore, we observed an equivalent decrease in CD49f/α6 integrin expression in all tumor models (data not shown). Reduced expression of CD29 and CD49f expression may be a feature of malignant progression during mammary oncogenesis and may occur to facilitate detachment of transformed MaSCs and basal progenitors from their microenvironment. Alternatively, the mammary tumors arising in these models may originate directly from the $CD29^{lo}CD24^+$ luminal population.

Determination of the frequency of tumorigenic cells in mouse mammary cancers. To determine the frequency of tumorigenic cells for each model, we transplanted Lin^- cells in decreasing numbers and performed limiting dilution analysis. The tumorigenic cell frequency for MMTV-wnt-1 tumors was 1/177 [95% confidence interval (95% CI), 1/246–1/127] based on at least three tumors, although variation between individual tumors was seen. Thus, we observed a substantially higher tumorigenic cell frequency in the MMTV-wnt-1 tumors than the recently

[3] http://bioinf.wehi.edu.au/software/limdil/index.html
[4] http://www.r-project.org/

[5] Unpublished data.

reported 1 in 7,496 cells (13), perhaps reflecting different transplantation sites. In MMTV-neu tumors, the frequency was 1/112 (95% CI, 1/166–1/76). In BALB/c-p53$^{+/-}$–derived tumors, the frequency of tumorigenic cells was substantially less than in the MMTV long terminal repeat–driven models (1/1,090; 95% CI, 2,030–1/580). It is notable that cell sorting has an effect on the tumorigenic frequency, probably through loss of viability, as we observed a frequency of 1/61 (95% CI, 1/173–1/22) for unsorted cells versus 1/369 (95% CI, 1/1,217–1/112) for sorted cells from MMTV-neu tumors (all tumor cells including Lin$^+$). CD24$^-$ cells did not give rise to tumors on transplantation and are likely stromal fibroblasts (2, 3). Although injection of tumor

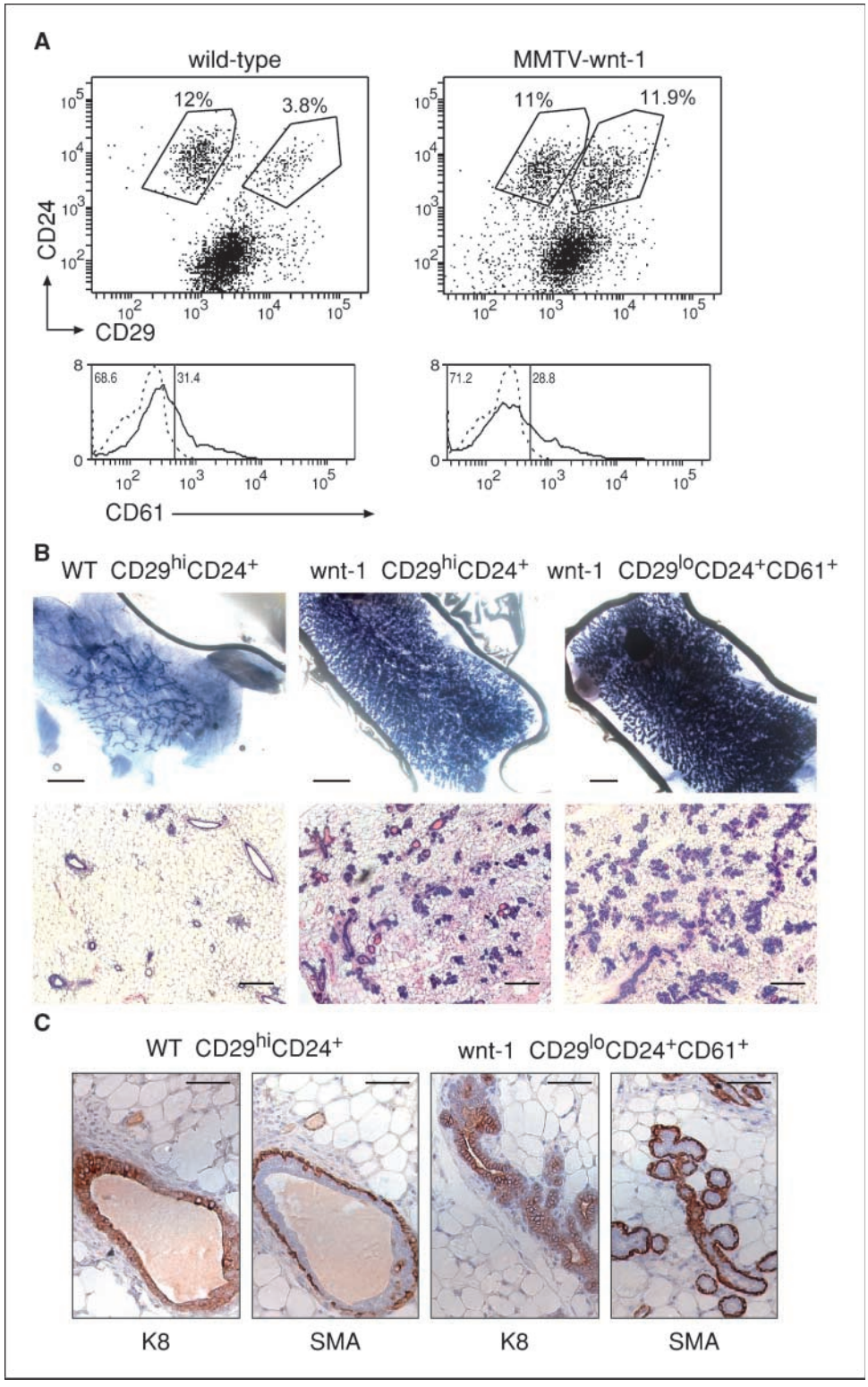

Figure 1. Generation of hyperplastic outgrowths from the stem cell–enriched and luminal fractions isolated from MMTV-wnt-1 preneoplastic mammary tissue. *A,* representative FACS dot plots (*top*) showing the expression of CD29 and CD24 in the CD45$^-$CD31$^-$ (Lin$^-$) population of mammary glands from 3-mo-old MMTV-wnt-1 and aged-matched wild-type mice. *Bottom,* expression of CD61 in the CD29^{lo}CD24$^+$ population. *Dashed line,* labeling with an isotype control antibody. *B,* whole mount (*top*) and H&E histologic (*bottom*) analyses of outgrowths (harvested at 8 wk) following transplantation of CD29^{lo}CD24$^+$ cells isolated from 8-wk-old wild-type mammary glands (100 cells) or CD29^{lo}CD24$^+$CD61$^+$ cells from MMTV-wnt-1 glands (200 cells). *Scale bars,* whole mounts, 2 mm (*left* and *middle*) or 1 mm (*right*); H&E, 200 μm. *C,* immunohistochemical analysis of primary outgrowths from CD29^{hi}CD24$^+$ cells taken from wild-type mice (*left*) or CD29^{lo}CD24$^+$CD61$^+$ cells isolated from preneoplastic MMTV-wnt-1 mammary tissue (*right*). *Left,* K8; *right,* SMA. *Scale bar,* 50 μm.

Table 1. Frequency of repopulating cells in preneoplastic MMTV-wnt-1 cell subsets

Type of cell transplanted	No. cells injected per fat pad	No. positive outgrowths*	
		Wild-type	MMTV-wnt-1
$CD29^{hi}CD24^{+}$	100	6/19	—
	200	10/18	15/19
	400	—	18/18
Repopulating frequency (95% CI)		1/253 (1/417–1/154)	1/111 (1/175–1/71)
		$P < 0.05$	
$CD29^{lo}CD24^{+}CD61^{+}$	50	—	1/13
	100	0/9	3/19
	200	0/6	3/6
Repopulating frequency (95% CI)		<1/700	1/464 (1/970–1/222)
		$P < 0.01$	
$CD29^{lo}CD24^{+}CD61^{-}$	300	—	3/19
	400	0/9	—
	600	—	2/19
Repopulating frequency (95% CI)		<1/1,325	1/3,210 (1/7,750–1/1,330)
		$P = 0.15$	

NOTE: The repopulating frequency was calculated by limiting dilution analysis as described (2). Double-sorted cells from preneoplastic tissue (2–3 mo of age) of wild-type or MMTV-wnt-1 glands were transplanted into the cleared mammary fat pads of 3-wk-old syngeneic (BALB/c) recipients. Data are pooled from three independent experiments.
*Shown as number of outgrowths per number of injected fat pads.

cells in Matrigel dramatically increased tumorigenicity (data not shown), we performed transplants without this matrix to avoid possible growth- and/or survival-promoting effects on tumor cells.

CD61 identifies CSCs in MMTV-wnt-1 but not MMTV-neu mammary tumors. Using the luminal progenitor marker CD61, a small $CD61^{+}$ fraction was distinguishable in MMTV-wnt-1 tumors ($n = 5$ tumors; Fig. 2*B*). The $CD29^{lo}CD24^{+}CD61^{+}$ population was significantly enriched for tumor-initiating cells compared with the $CD29^{lo}CD24^{+}CD61^{-}$ population (Supplementary Table S2) and could recapitulate a tumor that exhibited a similar immunophenotype (Fig. 2*B*) and histologic appearance to the original tumor. Furthermore, these tumors could be serially passaged. Only $CD29^{lo}CD24^{+}CD61^{+}$ cells from secondary tumors could regenerate a tumor (Fig. 2*B*) and at a comparable frequency to those derived from primary tumors (Supplementary Table S2). Moreover, transplantation of $CD29^{lo}CD24^{+}CD61^{+}$ cells from a secondary tumor generated tertiary tumors with a similar cell surface phenotype (Fig. 2*B*). Immunostaining showed that both primary and secondary wnt-1 tumors expressed luminal as well as myoepithelial markers (Fig. 2*B, bottom*), consistent with the notion that these originate from a bipotential progenitor (20). It is noteworthy that cells displaying the highest expression of CD29 also expressed abundant CD61 (Fig. 2*C*).

In contrast, all epithelial cells within MMTV-neu tumors were found to express very high levels of CD61 (Fig. 2*D*), consistent with their luminal gene profile (21). Using antibodies against several markers, including CD90, CD18, CD14, and Sca-1, we were unable to subdivide the $CD29^{lo}CD24^{+}$ population in MMTV-neu tumors (data not shown). Little expression of Sca-1 was observed in MMTV-neu tumors, consistent with the findings of Liu and colleagues (22). Furthermore, there seemed to be no difference in the cell cycle distribution or tumorigenic frequency of different Hoechst-stained fractions (data not shown).

Evidence for CSCs in some but not all mammary tumors arising in $p53^{+/-}$ mice. In the $p53^{+/-}$ model, tumors arise spontaneously with a latency of ~12 months. Although a broad peak of CD61 expression was apparent in primary $p53^{+/-}$ tumors, in three of six tumors CD61 led to definition of a $CD29^{lo}CD24^{+}CD61^{+}$ population that had considerably higher tumorigenic potential. Approximately 400 cells from this subset (arbitrarily designated type I tumors; Fig. 3*A*) were sufficient to generate a tumor, resulting in a 6.6-fold enrichment in tumor-forming ability relative to the $CD61^{-}$ population ($CD61^{+}$, 1/431 versus $CD61^{-}$, 1/2,800; Supplementary Table S3). Intriguingly, in the other three tumors (designated type II; Fig. 3*B*), there were no differences in tumor-initiating capacity between the $CD61^{+}$ and $CD61^{-}$ populations. However, these proved to have a substantially higher tumorigenic frequency (ranging from 10- to 22-fold) than the type I $p53^{+/-}$ tumors. Interestingly, a distinct pattern emerged for the different types of tumor on serial passaging. Augmented CD61 expression was evident in secondary and tertiary tumors generated from the type I $p53^{+/-}$ tumor. Conversely, secondary tumors arising from primary type II tumors exhibited down-regulation of CD61 expression. The heterogeneity seen in the $p53^{+/-}$ tumors presumably relates to the long latency with which these tumors arise, with the accumulation of different mutations leading to tumors that are sustained by different mechanisms. Indeed, gene expression profiling of tumors harboring loss of p53 (either heterozygous or homozygous) has indicated that they are heterogeneous (21). No notable differences in luminal or myoepithelial marker expression were observed between the two tumor types (Fig. 3*C*). The mechanisms underlying these tumorigenic differences have yet to be elucidated but are likely to involve distinct molecular pathways.

Discussion

We show that constitutive wnt-1 signaling in the preneoplastic stage perturbs the epithelial hierarchy, leading to the emergence of aberrant multipotential stem-like cells in the committed luminal cell fractions. This may reflect wnt-1 conferring stem-like properties on progenitors as they transit through a bipotent state or some diminution of CD29 expression in the MaSC population during wnt-1–induced transformation. Previous studies have suggested that the oncogenic effects of wnt-1 on mammary epithelium are initiated in mammary progenitor cells (20, 23) and wnt-1/β-catenin has recently been implicated in conferring radiation resistance on mammary progenitor cells (24). The enhanced self-renewal apparent in wnt-1 mammary glands is reminiscent of that occurring in other cellular compartments (25). Interestingly, in granulocyte-macrophage progenitors in chronic myeloid leukemia, activation of β-catenin has been linked to increased self-renewal (26).

We found that expression of the luminal mammary progenitor marker CD61 defined an enriched CSC population in specific mouse mammary tumors, giving a 7- to 20-fold enrichment of CSCs in $p53^{+/-}$ and MMTV-wnt-1 tumors, respectively. In the wnt-1 model, secondary tumors retained the hierarchical organization of the primary tumor, whereas in $p53^{+/-}$ tumors (type I), the proportion of $CD61^+$ cells increased on serial passaging, suggesting that the cells may be in a less differentiated state. The precise relationship between CD61 and Thy-1 expression (13) is not yet clear. Notably, there is evidence for CSCs in other mouse models of epithelial carcinogenesis. Cutaneous tumors arising from 7,12-dimethylbenz(a)anthracene/12-*O*-tetradecanoylphorbol-13-acetate–treated mice harbor a $CD34^+$ subpopulation that comprises CSCs. Significantly, wnt/β-catenin signaling was established to be essential for the maintenance of squamous CSCs, whereas normal epidermal homeostasis was unaffected by loss of this pathway (27).

The CSC model is unlikely to apply to all mouse and human tumors. In the case of MMTV-neu tumors, which display substantial homogeneity, a CSC population could not be defined using multiple different markers. Furthermore, CD61 did not resolve a CSC fraction in 50% of $p53^{+/-}$ mammary tumors nor in doxycycline-inducible Myc tumors, which are also relatively

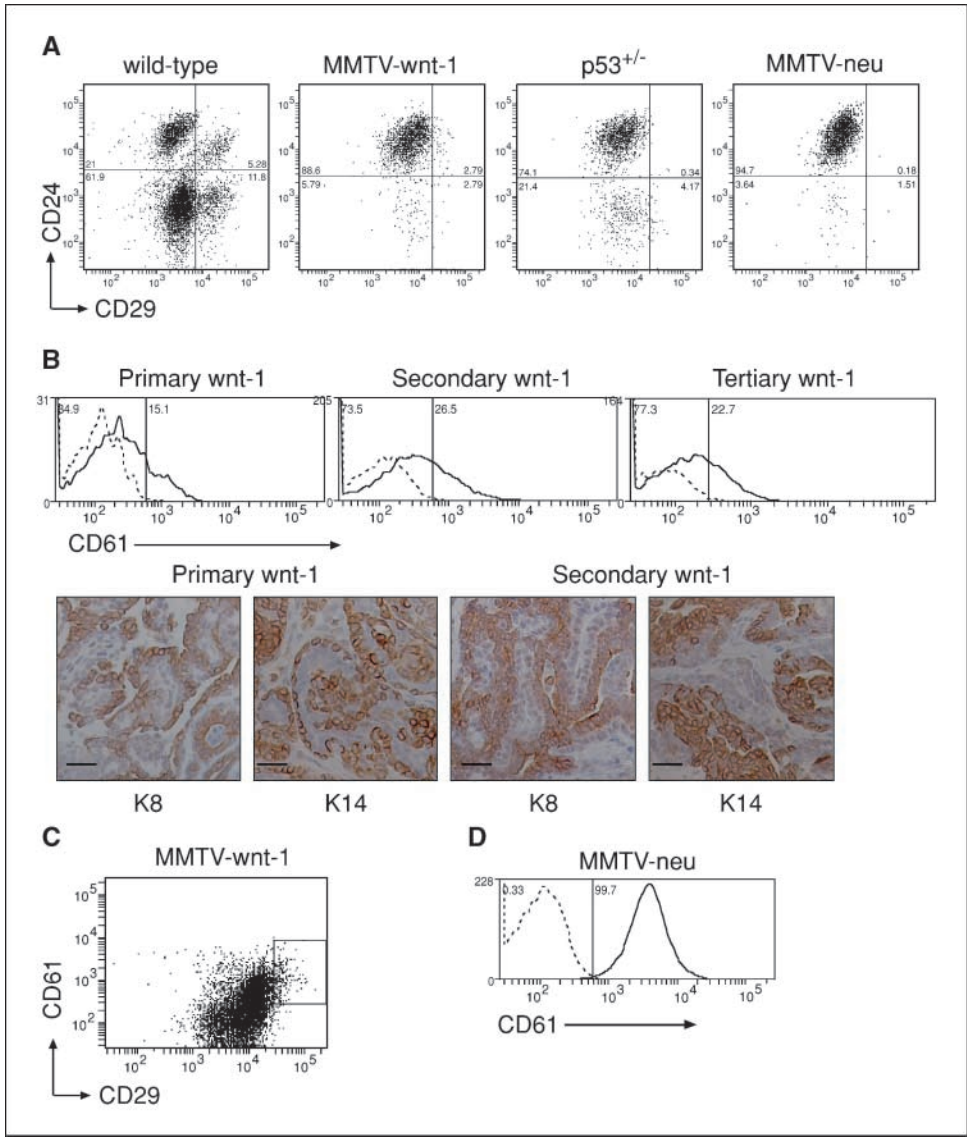

Figure 2. CD61 identifies CSCs in MMTV-wnt-1 mammary tumors. *A,* down-regulation of CD29 in tumor cells from MMTV-wnt-1, BALB/c-$p53^{+/-}$, and MMTV-neu mice. Representative FACS dot plots showing the expression of CD29 and CD24 in the $CD45^-CD31^-TER119^-$ (Lin^-) populations from wild-type, MMTV-wnt-1, MMTV-neu, and $p53^{+/-}$ mammary tumors. *B,* representative FACS histograms (*top*) showing the expression of CD61 in the $CD29^{lo}CD24^+$ populations of primary (*left*), secondary (*middle*), and tertiary (*right*) MMTV-wnt-1 tumors. *Dashed line,* isotype antibody control. Percentages of $CD61^-$ and $CD61^+$ cells are indicated. Immunohistochemical analysis (*bottom*) of primary (*left*) and secondary (*right*) wnt-1–induced tumors for K8 (*left*) and K14 (*right*). No staining was seen with control antibodies. *Scale bar,* 25 μm. *C,* representative FACS dot plot showing the expression of CD29 and CD61 in the Lin^-CD24^+ population of a MMTV-wnt-1 primary tumor. Square gate corresponds to the $CD29^+$ population. *D,* representative FACS histogram showing the expression of CD61 in the $Lin^-CD29^{lo}CD24^+$ population of a primary MMTV-neu tumor. *Dashed line,* isotype control. Percentages of $CD61^-$ and $CD61^+$ cells are indicated.

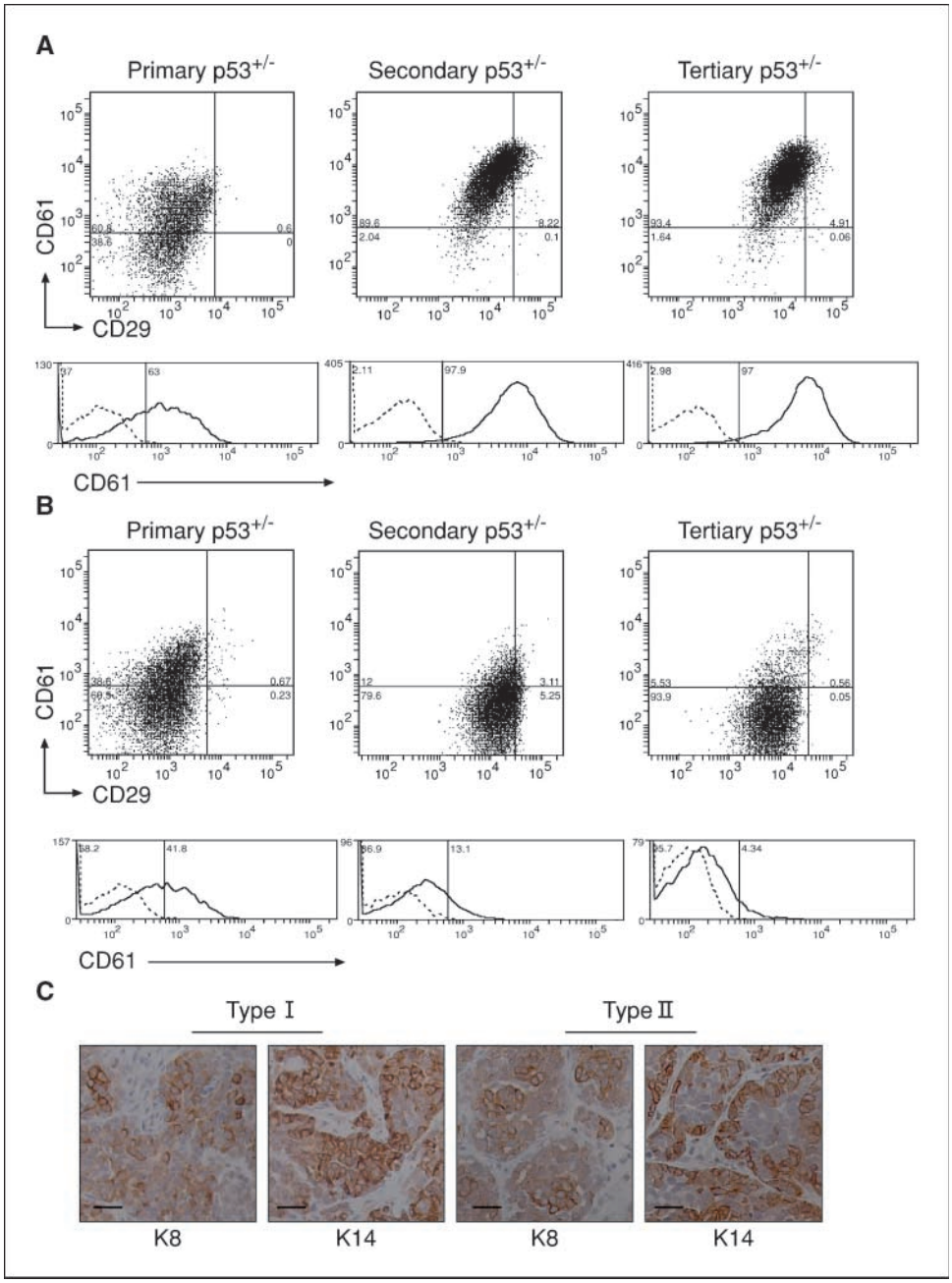

Figure 3. CD61 enriches for CSCs in some but not all $p53^{+/-}$ tumors. *A* and *B,* CD61 expression in $CD29^{lo}CD24^{+}$ cells from type I and II $p53^{+/-}$ tumors, respectively. Representative FACS dot plots showing the expression of CD29 and CD61 within the $Lin^{-}CD24^{+}$ population and histograms showing the expression of CD61 within the $Lin^{-}CD29^{lo}CD24^{+}$ population of $p53^{+/-}$ tumors of type I (*A*) or type II (*B*). Representative profiles of primary (*left*), secondary (*middle*), and tertiary (*right*) tumors are presented. *Dashed line,* isotype control. *C,* K8 and K14 immunostaining of type I (*left*) and type II (*right*) primary tumors. *Scale bars,* 25 μm.

homogeneous (data not shown). Although an alternative model (clonal evolution) may underlie tumorigenesis in these mice, we cannot exclude the CSC paradigm at this stage. In mouse models of hematopoietic malignancy that exhibit considerable homogeneity, a high frequency of tumorigenic cells was revealed. Kelly and colleagues (28) reported that >10% of tumor cells in B lymphomas of Eμ-myc transgenic mice, T lymphomas of Eμ-N-ras transgenic mice, and acute myelogenous leukemia developing in PU-1–deficient mice have tumor-forming ability in nonirradiated recipients. These hematopoietic malignancies may develop according to the clonal evolution model or, alternatively, a high proportion of CSCs could drive tumor growth.

The therapeutic relevance of CSCs has not yet been determined but the existence of CSCs has profound implications if they contribute to tumor relapse. In cell lines derived from *Brca1*-deficient mouse mammary tumors, heterogeneous CSC populations were evident and the putative CSCs were significantly more resistant to DNA-damaging drugs (29). In Brca1/p53-mediated mouse mammary tumors, cancer stem-like cells were recently implicated in cisplatin resistance (30), whereas in human breast tumors, there is clinical evidence for a subset of chemotherapy-resistant breast cancer–initiating cells (31). It will be important to determine whether CD61 can also identify CSCs in human breast cancer and their therapeutic relevance. It is notable that the αv/β3 integrin complex is a marker of poor prognosis in breast cancer and that it regulates breast tumor metastasis (14, 15). Moreover, the recent development of a humanized monoclonal antibody against the αv/β3 integrin complex shows considerable

promise in direct targeting of tumor cells that express this complex (32).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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