Dissecting the roles of β-catenin and cyclin D1 during mammary development and neoplasia

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A considerable body of circumstantial data suggests that *cyclin D1* is an attractive candidate to mediate the effects of β-catenin in mammary tissue. To test the functional significance of these correlative findings, we investigated the genetic interaction between transcriptionally active β-catenin (ΔN89β-catenin) and its target gene cyclin D1 in the mouse mammary gland during pubertal development, pregnancy, and tumorigenesis. Our data demonstrate that cyclin D1 is dispensable for the ΔN89β-catenin-stimulated initiation of alveologenesis in virgin females, for the *de novo* induction of alveoli in males, and for the formation of tumors. Indeed, lack of cyclin D1 accentuates and enhances these hyperplastic and tumorigenic ΔN89β-catenin phenotypes. Although alveologenesis is initiated by ΔN89β-catenin in a cyclin D1-independent fashion, up-regulation of cyclin D1 occurs in ΔN89β-catenin mice and its expression remains essential for the completion of alveolar development during the later stages of pregnancy. Thus, alveologenesis is a two-step process, and cyclin D1 activity during late alveologenesis cannot be replaced by the activity of other β-catenin target genes that successfully drive proliferation at earlier stages.

mammary tumor | Wnt | cell adhesion | cell cycle | cadherins

The protein β-catenin is a multifunctional intracellular protein. In adherent cells it links cadherin cell-adhesion molecules to the actin cytoskeleton, thus playing an important role in stabilizing cell–cell adhesion. Additional pools of β-catenin shuttle between the cytoplasm and the nucleus and regulate the transcriptional capabilities of T cell factor/lymphoid enhancement factor DNA-binding proteins (1).

β-Catenin's role in the canonical Wnt-signaling pathway has been investigated extensively, and recent studies have shown that it lies at the hub of several other major signaling pathways (ref. 1 and references therein). Many of these pathways stabilize β-catenin and enhance its transcriptional capability by preventing phosphorylation of the N-terminal domain. In the mammary gland, expression of N-terminally truncated, stabilized mouse mammary tumor virus (MMTV)-ΔN89β-catenin causes precocious alveolar development in virgin mice and delayed involution and development of mammary tumors (2). These data suggest that β-catenin signaling determines alveolar cell fate, survival, and/or proliferation. This concept is supported by the effects of overexpression of β-catenin activators (Wnt 1, 3, and 10b) and suppressors (axin and a dominant-negative β-catenin construct β-engrailed), which stimulate and impair alveolar development, respectively (3–5).

Of the multiple target genes of β-catenin signaling identified so far (www.stanford.edu/~rnusse/pathways/targets.html), the cell-cycle regulator cyclin D1 (referred to as cycD1 in the figures) has attracted particular interest. The phenotypic congruency among mice overexpressing or lacking the activity of cyclin D1 or β-catenin suggests a functional linkage between these proteins during normal mammary development. Specifically, both MMTV-ΔN89β-catenin and MMTV-cyclin D1 induce precocious mammary gland development and mammary tumors (2, 6). In addition, inappropriate up-regulation of cyclin D1 mRNA levels accompanies the various phenotypes induced in mice by MMTV-ΔN89β-catenin. Moreover, cyclin D1-null mice and mice expressing the negative regulators of β-catenin signaling axin or β-engrailed show similar impairments in alveolar development (3, 4, 7–9). Finally, in humans coincident elevations of β-catenin and cyclin D1 have been demonstrated in breast cancer and correlated with poor clinical outcome (10).

Collectively, these findings are consistent with, but do not prove, a functional connection between β-catenin signaling and cyclin D1 up-regulation in human and mouse mammary development and oncogenesis. To test the physiological relevance of these correlative findings, we investigated the effects of expressing stabilized β-catenin in the absence of its downstream target cyclin D1 in the mouse mammary gland. These studies reveal cyclin D1-independent effects of β-catenin during pubertal development and oncogenesis, but an indispensable requirement for cyclin D1 during pregnancy-induced alveologenesis that cannot be circumvented by other β-catenin target genes.

Materials and Methods

Mice. Transgenic MMTV-ΔN89β-catenin FVBN mice were crossed with cyclin $D1^{+/-}$ animals, and the resulting offspring were intercrossed, generating six genotypes (2, 8). One group of 60 female mice were kept as virgins, and a second set of female mice were mated at 40 days of age and bred continuously to enhance expression of the transgene. Mice were killed before the tumors reached 5% of body weight or at 260 days of age for the breeding group or at 380 days for the virgin group. Survival curves were analyzed statistically by using the Mann–Whitney *U* test. To analyze changes in the mammary gland that were induced by pregnancy, female mice were mated at 6 weeks of age and checked daily for vaginal plugs. The stage of pregnancy was confirmed by observing the stage of limb development in their embryos.

Whole Mounts, Histology, Oil Red O Staining, and Immunohistochemistry. Whole-mount and histological analysis were performed as described (2). For oil red O staining, 10-μm cryosections were fixed for 1 min in 40% formaldehyde and washed in tap water. Sections were stained for 10 min at room temperature in oil red O (0.06% oil red O/62.5% isopropyl alcohol), washed in water, and counterstained in hematoxylin. For immunohistochemical analysis, antigen retrieval, staining with mouse monoclonal anti-proliferating cell nuclear antigen (1:200, DAKO), and detection by the Animal Research Kit (DAKO) were carried out according to the manufacturer's instructions. For the rabbit polyclonal anti-casein serum (1:100), we used the EnVision+ System, peroxidase anti-rabbit IgG (DAKO), followed by diaminobenzidine.

Abbreviation: MMTV, mouse mammary tumor virus.

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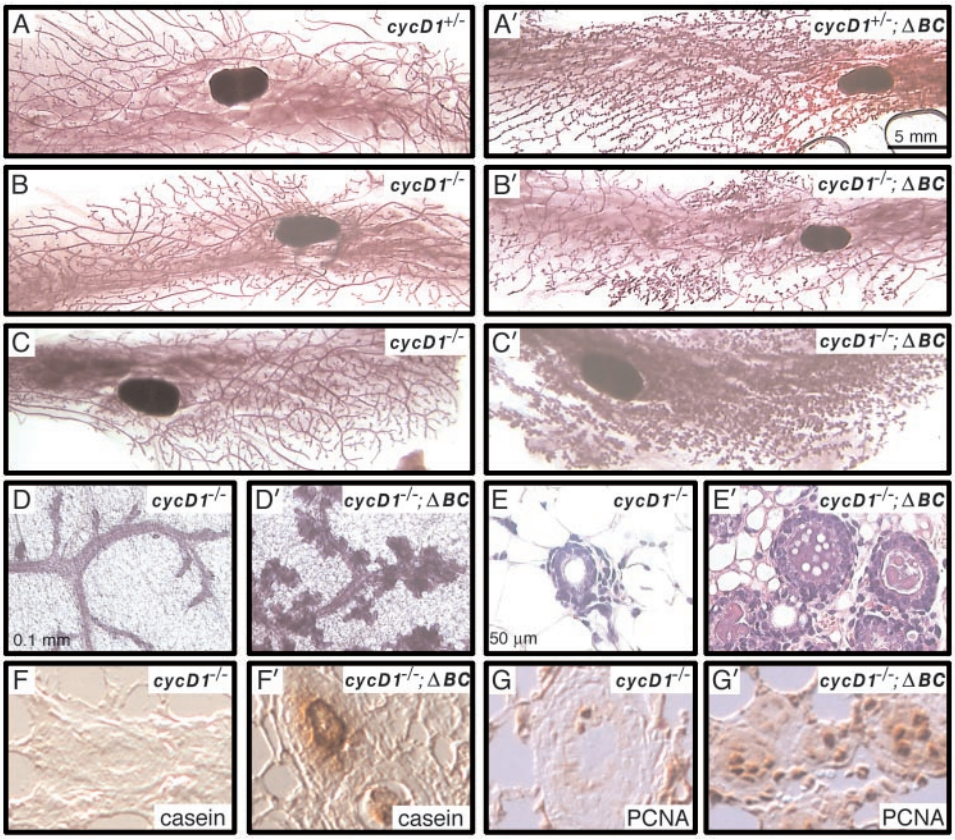

Fig. 1. ΔN89-β-catenin (ΔBC) induces precocious mammary development in virgin female mice in the absence of cyclin D1. Shown are wholemounts (*A–D* and *A′–D′*) and histological sections (*E–G* and *E′–G′*) of mammary glands from 12-week (*A*, *A′*, *B*, and *B′*) and 26-week (*C–G* and *C′–G′*) virgin female littermates stained with hematoxylin and eosin (*E* and *E′*), anti-casein (*F* and *F′*), and anti-proliferating cell nuclear antigen (anti-PCNA) (*G* and *G′*) antibodies.

Western Blot Analysis. Total protein extracts of mammary gland and Western blot analysis were carried out as described (2) by using primary mouse antibodies against cyclin D2, cyclin D3 (both 1:200, NeoMarkers, Fremont, CA), or E-cadherin (1:4,000, BD Transduction Laboratories, Lexington, KY), or rabbit polyclonal antibodies against cyclin E (1:200), c-myc (1:500), β-catenin (1:4,000) (Santa Cruz Biotechnology), or sheep anti-cytokeratin 8 (1:1,000, PickCell Laboratories, Leiden, The Netherlands). Secondary antibodies were anti-mouse, anti-rabbit (both 1:4,000, Amersham Pharmacia), or anti-sheep (1:2,000, ICN) conjugated to horseradish peroxidase and visualized by an enhanced chemiluminescence system (Amersham Pharmacia).

Results

ΔN89β-Catenin Does Not Require Cyclin D1 to Induce Precocious Alveologenesis in Virgin Mammary Glands. To test the physiological relevance of the cyclin D1 elevation seen in response to β-catenin signaling in the mammary gland (2), we compared the phenotypes of cyclin $D1^{+/+}$, cyclin $D1^{+/-}$, and cyclin $D1^{-/-}$ littermates expressing the MMTV-ΔN89β-catenin transgene. As expected, MMTV-ΔN89β-catenin induced small spherical outgrowths, reminiscent of alveolar structures, along the mammary ducts of virgin cyclin $D1^{+/+}$ and cyclin $D1^{+/-}$ mice (Fig. 1*A′*). Surprisingly, although cyclin $D1^{-/-}$ mice are reported to be impaired in alveologenesis (8, 9), the ΔN89β-catenin transgene successfully induced precocious development in female cyclin $D1^{-/-}$ ΔBC mice (Fig. 1*B′*). This development occurred in a focal fashion in young adult mice but became more generalized with age (Fig. 1 *C′* and *D′*). cyclin D1-null mice are runted early in life but eventually attain a normal size. These early phenotypes therefore represent the effects of an enhanced hyperplasia in response to the expression of the transgene superimposed on a retarded pubertal development. Histological analysis showed that these structures resemble alveoli, as judged by the presence of fat droplets within the mammary epithelial cells and luminal secretions (Fig. 1*E′*) that stained positively with anti-casein antibodies (Fig. 1*F′*). MMTV-ΔN89β-catenin induced expression of proliferating cell nuclear antigen, a marker of cell proliferation, regardless of the lack of cyclin D1 (Fig. 1*G′*). These data indicate that ΔN89β-catenin initiates alveologenesis in a cyclin D1-independent fashion and at this stage can provoke a robust proliferative response in the absence of cyclin D1.

Large, flowery hyperplasias were also observed, expanding into more than half of the fat pad, in transgenic cyclin $D1^{-/-}$ΔBC males (Fig. 2 *A′* and *B*), that contained secretions (Fig. 2*C*), which stained positively for fat with oil red O (Fig. 2*D*) and contained casein (Fig. 2*E*). These features resemble those found in alveolar structures around 6–10 days of pregnancy. Thus, ΔN89β-catenin can induce alveolar differentiation and the expression and secretion of milk proteins in male and female mammary glands in the absence of upstream hormonal stimuli elicited by pregnancy or estrus and in the absence of its downstream target gene cyclin D1.

ΔN89β-Catenin Cannot Rescue Defects in Alveolar Expansion in the Mammary Glands of Pregnant cyclin D1-Null Mice. During the course of pregnancy, a rapid alveolar expansion occurs in cyclin $D1^{+/+}$ and cyclin $D1^{+/-}$ mice. Mammary glands from 12-days (Fig. 3 *A*, *A′*, *B*, and *B′*) and 17-days pregnant (Fig. 3 *C*, *C′*, *D*, and *D′*) cyclin $D1^{+/+}$ mice exhibit little difference between nontransgenic and β-catenin-transgenic groups. Under the hormonal

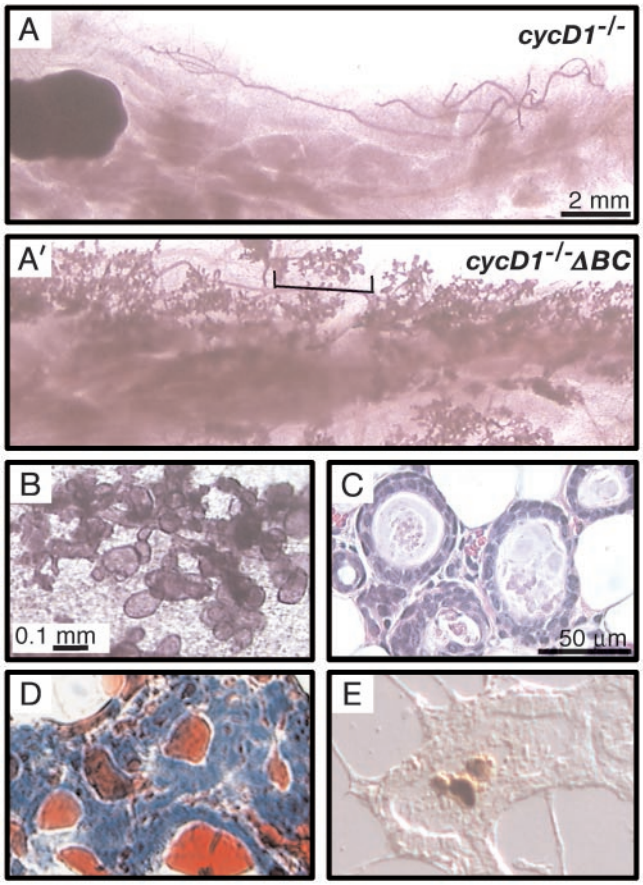

Fig. 2. ΔN89β-catenin induces alveolar development and milk-protein expression in male mammary glands in the absence of cyclin D1. Shown are wholemounts (*A*, *A′*, and *B*) and histological sections of mammary glands from 20-week-old cyclin D1$^{-/-}$ (*A*) and cyclin D1$^{-/-}$ΔBC (*A′*–*E*) male mice stained with hematoxylin and eosin (*C*), oil red O (*D*), and anti-casein antibodies (*E*).

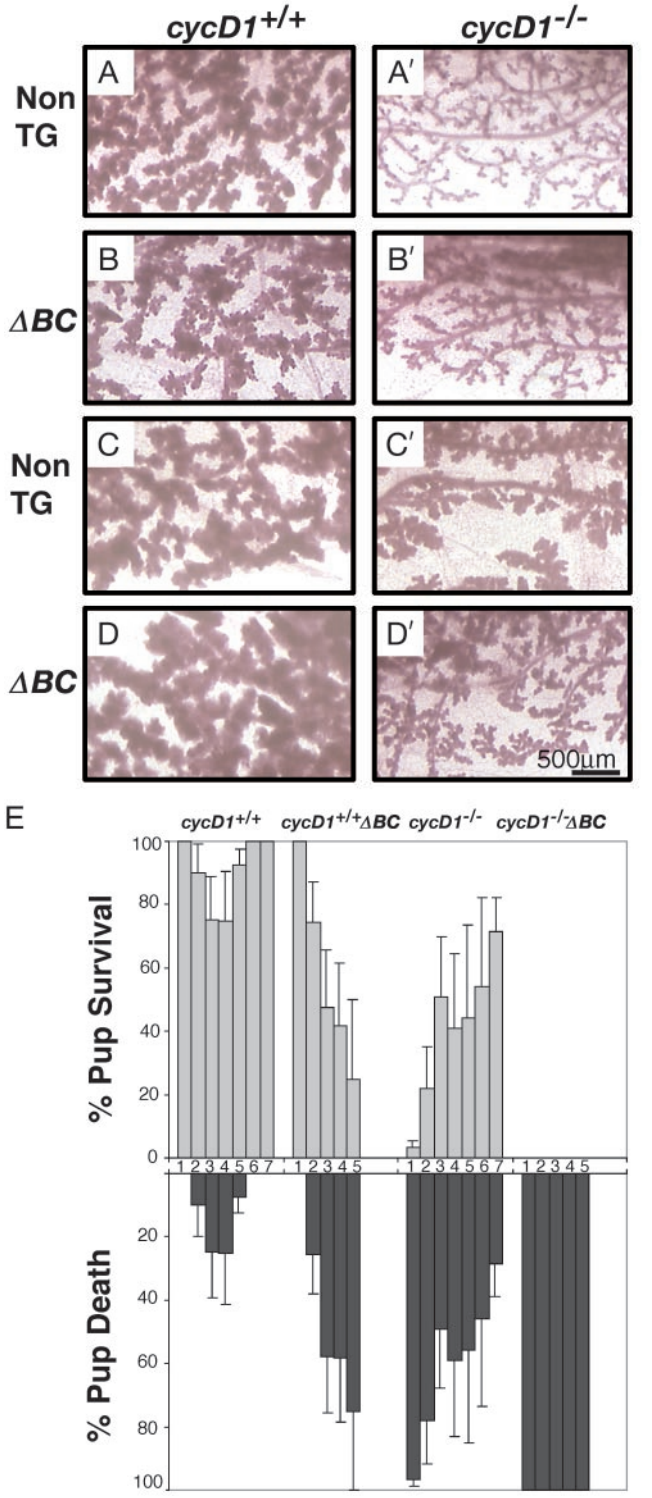

Fig. 3. ΔN89β-catenin expression does not overcome the cyclin D1-null defects in pregnancy-induced alveologenesis. Shown are wholemounts of mammary glands from 12.5-day (*A*, *B*, *A′*, and *B′*) and 17.5-day (*C*, *D*, *C′*, and *D′*) pregnant cyclin D1$^{+/+}$ (*A*–*D*) and cyclin D1$^{-/-}$ (*A′*–*D′*) mice in the presence (ΔBC) or absence (Non TG) of the transgene. (*E*) The mean percentage of pups surviving (□) or dying (■) in each of seven litters is plotted for each genotype. Error bars represent SEM.

stimulation of pregnancy, nontransgenic mice essentially "catch up" to the stage of development that occurred precociously in their transgenic littermates. Intriguingly, given the ability of β-catenin to provoke precocious development in the absence of cyclin D1 and to stimulate several additional genes that promote cell proliferation, both nontransgenic (Fig. 3 *A′* and *C′*) and transgenic (*B′* and *D′*) cyclin D1$^{-/-}$ littermates remained blocked in alveolar expansion.

This developmental block was further demonstrated by an analysis of the survival of pups from mothers of differing cyclin D1 genotypes over five to seven consecutive litters (some groups ceased to breed earlier because of tumor load) (Fig. 3*E*). Equal numbers of pups were born to transgenic mice regardless of their cyclin D1 status (an average of 6.3, 6.3, and 6.6 for cyclin D1$^{+/+}$, cyclin D1$^{+/-}$, and cyclin D1$^{-/-}$ groups, respectively), yet pup survival differed in a statistically significant way among these groups. Notably, 100% of pups born to transgenic cyclin D1$^{-/-}$ mothers fail to survive to weaning age, demonstrating a statistically significant accentuation of the ΔN89β-catenin phenotype in the absence of cyclin D1 ($P \leq 0.005$ when compared with pup survival in cyclin D1$^{+/+}$ΔBC litters 1 and 2). This finding contrasts to the milder phenotypes seen in nontransgenic cyclin D1$^{-/-}$ mothers that cannot nurse the first litter but show progressive improvement with subsequent litters ($P < 0.002$ compared with cyclin D1$^{+/+}$ mothers during litters 1 and 2, but no significant value thereafter), and also with cyclin D1$^{+/+}$ΔBC mice that nurse the first litter but deteriorate in their ability to raise subsequent litters (first vs. second litter = $P < 0.05$; first vs. third = $P < 0.003$; first vs. fourth = $P < 0.0007$; first vs. fifth = $P < 6 \times 10^{-5}$) (2, 8, 9). From these data we draw two conclusions.

First, the ΔN89β-catenin phenotype is strikingly accentuated in cyclin D1$^{-/-}$ mice. Second, ΔN89β-catenin cannot direct the expansion of a fully functional mammary gland in the absence of the target gene cyclin D1. Thus, other ΔN89β-catenin target genes cannot compensate for cyclin D1 activity in the pregnant mammary gland.

ΔN89β-Catenin Can Induce Tumors Independently of Cyclin D1. As cyclin D1 is a target gene of β-catenin signaling, it is possible that it is an essential mediator of β-catenin-induced mammary oncogenesis (10, 11). To address this issue, we compared the propensity of MMTV-ΔN89β-catenin to induce tumors in cyclin D1$^{+/+}$ and cyclin D1$^{-/-}$ littermates. No significant difference was found between cyclin D1$^{+/+}$ΔBC or cyclin D1$^{+/-}$ΔBC groups, so the data from these groups have been combined. Our previous work has demonstrated that ΔN89β-catenin induces tumors at ≈4 months of age in breeding mice and at ≈7 months in virgin mice on an FVBN strain background, and similar results were observed in this study for the combined cyclin D1$^{+/+}$ΔBC/cyclin D1$^{+/-}$ΔBC cohorts (2) (Fig. 4*A*). Unexpectedly, ΔN89β-catenin-induced mammary tumors in cyclin D1$^{-/-}$ΔBC mice with increased incidence in comparison with the cyclin D1$^{+/+}$ΔBC/cyclin D1$^{+/-}$ΔBC group (Fig. 4*A*). The median time for the ΔN89β-catenin transgene to elicit mammary tumors for breeding mice was 186 days for cyclin D1$^{+/+}$ΔBC/cyclin D1$^{+/-}$ΔBC mice and 146.5 days for cyclin D1$^{-/-}$ΔBC mice. Statistical analysis (Mann–Whitney *U* test) of the survival curves showed a significant difference between the cyclin D1$^{+/+}$ΔBC/cyclin D1$^{+/-}$ΔBCs and cyclin D1$^{-/-}$ΔBC groups ($P < 0.002$). Virgin mice developed mammary tumors with a similar pattern of incidence, but with longer latency because of decreased expression of the MMTV transgene (Fig. 4*A*). cyclin D1$^{-/-}$ΔBC mice exhibited an increased number of tumors (Fig. 4*B*) and an increased number of involved mammary glands per mouse (Fig. 4*C*) as compared with their cyclin D1$^{+/+}$ΔBC and cyclin D1$^{+/-}$ΔBC littermates ($P < 0.002$ for tumors, $P < 0.03$ for glands). No tumors were observed in nontransgenic mice. Both the developmental and tumorigenic phenotypes described above showed full penetrance and thus were not subject to the effects of modifier genes found in mixed-background mice. Histological analysis revealed no obvious difference in the adenocarcinomas produced by different cyclin D1 genotypes (data not shown). These results indicate that although ΔN89β-catenin can and does up-regulate cyclin D1 protein levels in the tumors that it induces, β-catenin-driven tumorigenesis can proceed independently of cyclin D1 activity in the mammary gland. Indeed, the absence of cyclin D1 leads to increased tumor incidence and frequency, suggesting that its activity may act as a brake on β-catenin-induced mammary neoplasia.

To investigate the mechanism by which ΔN89β-catenin drives mammary tumorigenesis in the absence of cyclin D1, Western blot analysis was performed on other proteins important for orderly cell-cycle progression (Fig. 4*D*). Protein levels of the oncogene and β-catenin target c-myc were increased in the majority of tumors from transgenic mice, compared with uninvolved glands, regardless of cyclin D1 status. Cyclins D2, D3, and E were detected in lysates from some mammary glands and tumors, but were not up-regulated in a consistent manner in cyclin D1$^{-/-}$ΔBC tumors. All tumors continued to express the ΔN89β-catenin transgenic protein.

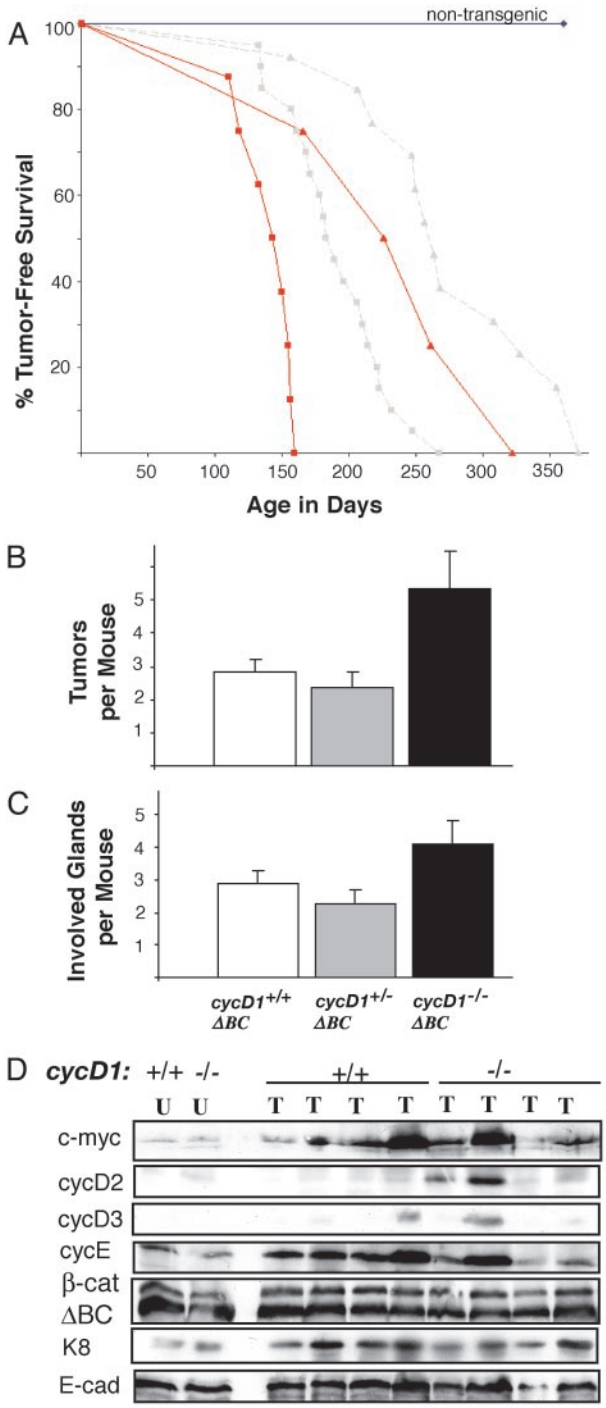

Fig. 4. Mammary tumor formation is enhanced in cyclin D1$^{-/-}$ΔBC mice. (*A*) cyclin D1$^{-/-}$ΔBC mice (red lines) develop tumors faster than transgenic cyclin D1$^{+/+}$ΔBC or cyclin D1$^{+/-}$ΔBC littermates combined (gray dashes). Virgin mice (▲) show the same trend as breeding mice (■) but take longer to manifest the phenotype. The mean number of tumors per mouse (*B*) and the mean number of involved mammary glands per mouse (*C*) are plotted for the various genotypes as indicated. Error bars represent SEM. (*D*) Western blots of total proteins from uninvolved mammary glands (U) and tumors (T) from cyclin D1$^{+/+}$ΔBC and cyclin D1$^{-/-}$ΔBC mice probed with antibodies against the indicated proteins. K8, cytokeratin 8; E-cad, E-cadherin.

Discussion

To test definitively whether cyclin D1 is an essential effector of β-catenin signaling in mammary gland development and cancer, we evaluated the consequences of expressing MMTV-ΔN89β-catenin in the absence of cyclin D1. Our data show that stabilization of β-catenin within the mammary ductal cell population initiates alveologenesis, stimulates milk-protein expression and secretion, and induces mammary tumors in the absence of cyclin D1. Despite this remarkable ability of β-catenin to initiate precocious and *de novo* alveologenesis in virgin and male

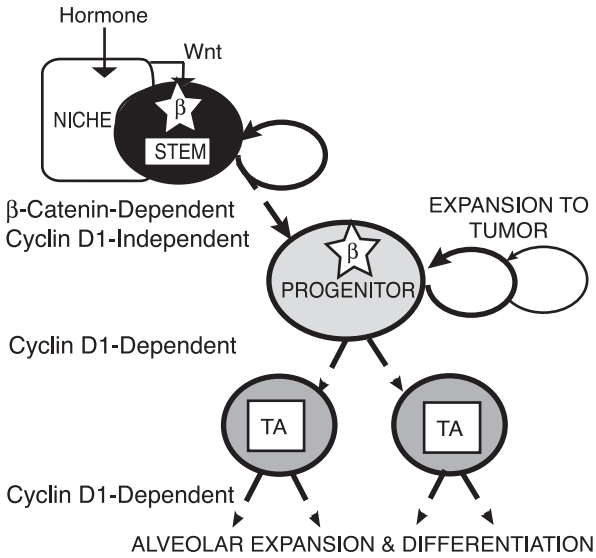

Fig. 5. A model of the role of β-catenin signaling in the regulation of mammary alveologenesis. Hormonal signals are received by receptor-bearing cells, which are envisaged to represent a stem cell niche (NICHE), and converted to paracrine Wnt signals encrypting side-branching and alveolar instructions. Receipt of Wnt signals in neighboring stem cells induces a β-catenin-dependent, cyclin D1-independent asymmetric division resulting in the regeneration of the stem cell and the production of an alveolar progenitor (Progenitor) that is casein-positive and depends on β-catenin (β) for survival. Cyclin D1-dependent divisions of this cell during pregnancy produce a lineage of ''transit-amplifying'' cells (TA) that, while expansive, undergo terminal differentiation during involution. Continuous activation of β-catenin signaling (β) would result in expansion of the stem/progenitor cell compartment, and would lead to hyperplasia and tumor formation, which would be further accentuated in the absence of cyclin D1 by the failure to convert these cells into a terminally differentiating population. Dashed arrows represent lineages that may encompass multiple cell divisions.

mice in the absence of cyclin D1, the requirement for cyclin D1 for the completion of alveolar expansion during later stages of pregnancy cannot be circumvented. Thus, alveologenesis involves at least two stages (Fig. 5). Early divisions in the alveolar lineage involve stimulation of a β-catenin target that is not cyclin D1, whereas later divisions, and possibly further differentiation, depend completely on cyclin D1, which is up-regulated in response to β-catenin (2). The findings that mice expressing suppressors of β-catenin cannot sustain alveologenesis in cyclin $D1^{+/+}$ mice (3, 4) and that cyclin $D1^{-/-}$ glands cannot progress even when β-catenin levels are augmented indicate that both stimuli are essential and act sequentially during pregnancy (Fig. 5).

Cyclin D1 promotes cell-cycle progression by forming complexes with cdk4 and cdk6 that phosphorylate Rb and additionally sequester the p27 inhibitory protein, thereby activating the cyclin E/cdk2 complex (12). Thus, the inability of cyclin D1 null mice to feed their pups is thought to result from reduced proliferation, leading to insufficient numbers of alveolar cells by the completion of pregnancy (7). Our results show that, although β-catenin induces proliferation in the absence of cyclin D1 at other stages of mammary development, these target genes cannot substitute for, or bypass, cyclin D1 activity to complete alveologenesis during late pregnancy. Thus, some specific aspect of the cyclin D1-driven cell cycle is required to generate a second wave of alveolar progeny and/or to promote their differentiation (Fig. 5). One possibility is that other β-catenin target genes that promote cell cycling and proliferation are silenced during late pregnancy. Consistent with this, different stages of mammary development appear to use specific complements of cell-cycle genes (13). A second possibility is that cyclin D1 is essential for a differentiation step that is key to alveolar progression. Findings that loss of p27 or that substitution of cyclin E for cyclin D1 can rescue the neural and retinal cyclin D1-null phenotypes have promoted the concept that cyclin D1 functions solely to advance the cell cycle (14, 15). However, the rescue of the mammary gland phenotype in these mice was only partial (35%), suggesting that cyclin D1 has additional cdk-independent roles in this location. A role for cyclin D1 in regulating the activity of the estrogen receptor has been suggested (16, 17).

Several observations imply a direct linear signaling pathway between β-catenin and cyclin D1 in tumorigenesis. In mice, both MMTV-ΔN89β-catenin and MMTV-cyclin D1 induce adenocarcinomas, and the β-catenin tumors display up-regulation of cyclin D1 (2, 6). In humans, *CCND1,* the gene encoding cyclin D1, is amplified or up-regulated in many tumor types, including 40% of breast cancers (18–21). The absence of *CCND1* gene amplification or mutation in the remainder of these tumors implicates upstream regulators of cyclin D1 gene expression in the initiation of the oncogenic process (22, 23). Transcriptional regulation of cyclin D1 by β-catenin signaling has been demonstrated in colonic, cervical, and breast tumor cells, and nuclear localization of β-catenin has been correlated in breast tumors with cyclin D1 expression and poor clinical outcome (10, 11, 24). Despite this body of literature linking cyclin D1 up-regulation to the oncogenic effects of β-catenin, our results firmly show that β-catenin-induced mammary hyperplasia and tumor incidence occur in the absence of cyclin D1.

This observation complements a recent study demonstrating that cyclin D1 is superfluous for MMTV-Wnt-1-driven mammary tumor formation (25). Wnts can activate several signaling cascades and, as secreted proteins, affect many mammary cell types (26). Our findings clarify and extend this study to show directly that cyclin D1 is dispensable for the tumorigenic effects of the canonical β-catenin pathway within mammary ductal epithelia. Contrary to previous suggestions that cyclin D2 might compensate for lack of cyclin D1, we saw no consistent change in the protein levels of other cyclins in cyclin $D1^{-/-}$ΔBC tumors. However, c-myc, which is a direct β-catenin target in colon cancer cells, is elevated in MMTV-ΔN89β-catenin tumors regardless of their cyclin D1 status. MMTV-c-myc also induces tumors in a cyclin D1-independent fashion (25). Taken together these results suggest that a direct linear pathway from Wnts via activated β-catenin and c-myc provides a cyclin D1-independent route to tumorigenesis in the mammary gland.

The observation that MMTV-ΔN89-β-catenin phenotypes are accentuated rather than impaired by loss of cyclin D1 was particularly surprising. The frequency of tumor incidence is increased, the total number of tumors and the number of glands affected are increased, the pup death rate is higher, and the precocious development is more pronounced in MMTV-ΔN89-β-catenin cyclin $D1^{-/-}$ mice. This observation raises the possibility that, although cyclin D1 elevation accompanies and is likely to be induced by β-catenin activation, it may nevertheless serve to provide a negative feedback on other β-catenin-stimulated proliferative pathways. In this respect, cyclin D1 appears to be acting differently in mammary glands than in intestine where loss of cyclin D1 reduces the tumorigenicity of APC^{min} (27). Although cyclin D1 is generally considered an accelerator of the G_1 phase, it has been reported to act as a brake to the S phase (28, 29) and to promote the maintenance of the differentiated state (30).

In the context of the emerging literature on β-catenin, the developmental and tumor phenotypes of the MMTV-ΔN89β-catenin/cyclin $D1^{-/-}$ mice lead us to propose a model in which β-catenin induces the production of alveolar progenitors. Precedence for the role of β-catenin in expanding the progenitor cell compartment exists in other tissues (31, 32). The hyperplasia

induced by β-catenin resembles changes in the mammary gland that accompany the hormonal surges of estrus, suggesting that MMTV-ΔN89β-catenin tumors may arise from locking the mammary gland into a state of "perpetual estrus," resulting in the rapid accrual of alveolar progenitors. In the second step, β-catenin induction of a cyclin D1-mediated cell cycle during pregnancy converts the alveolar progenitor into a pair of transit-amplifying daughters that proliferate extensively but are removed at the end of lactation (Fig. 5). Thus, lack of cyclin D1 expression would enhance tumor formation by resulting in a failure to clear progenitors. This model predicts that β-catenin and cyclin D1 may play significant roles in the known association between numbers of estrus and risk of breast cancer and with the protective effects of pregnancy. Finally, our results suggest that the targeting of cyclin D1 as a mammary cancer therapeutic strategy, although attractive because of its nonessential function in many tissues, could lead to deleterious effects, as tumors engendered by other proliferative pathways may progress faster in its absence.

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