Integrins in mammary-stem-cell biology and breast-cancer progression – a role in cancer stem cells?

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Summary

Cancer cells with stem cell-like properties (cancer stem cells) are believed to drive cancer and are associated with poor prognosis. Data from mouse models have demonstrated that integrins, the major cellular receptors for extracellular-matrix components, have essential roles both during cancer initiation and progression, and during cell differentiation in normal development. By presenting an overview of the role of integrins in stem-cell biology and in cancer progression, this Commentary aims to present evidence for a role of integrins in the biology of cancer stem cells. Given the recent interest in the role of integrins in breast-cancer initiation and progression, we focus on the role of the members of the integrin family and their coupled signaling pathways in mammary-gland development and tumorigenesis.

Key words: Breast cancer, Cancer stem cells, Integrin, Stem cells

Introduction

Integrins are a family of transmembrane proteins that, in humans, include 18 α- and 8 β-subunits (Giancotti and Tarone, 2003). Each β-subunit binds to one of several α-subunits and forms a heterodimeric receptor that is able to detect the glycoproteins that compose the extracellular matrix – either in basement membranes or in the interstitial matrix – or other proteins that are present on the surface of neighbouring cells. Hence, integrins constitute the major receptors for the environment of the cell. In addition to sensing the environment through their extracellular region, integrins are also able to engage several molecular effectors on their cytosolic side [these are described in detail elsewhere in this issue (Harburger and Calderwood, 2009; Legate and Fässler, 2009)]. Through their coupling to kinases, scaffolding proteins or small GTPases, integrins modulate an impressive panel of intracellular signaling pathways that determine the adhesion, migration, polarity, survival, growth or death status of the cell (Giancotti and Tarone, 2003). Not surprisingly, β1 integrin – which is able to associate with at least 12 of the 18 α-subunits (van der Flier and Sonnenberg, 2001) and binds to all the major components of the extracellular matrix – has been shown to be essential for normal mammalian development (its genetic deletion being embryonic lethal in mice) (Fassler and Meyer, 1995).

Given the longstanding knowledge that integrins are implicated in cancer-cell biology (Plantefaber and Hynes, 1989), the molecular mechanisms that allow integrins to promote cell proliferation and migration have been studied in great detail over the last two decades (Giancotti and Tarone, 2003). Although a number of seminal studies have shown that integrin expression is required to promote hyperproliferation and carcinogenesis in various types of epithelial cell (Guo and Giancotti, 2004; Watt, 2002) and is involved in the migration of metastatic cancer cells, the role of integrins in the early events of cancer progression has only recently been demonstrated. Indeed, the loss of function of β1 or β4 integrin, as well as some of their signaling partners, in transgenic mouse models of breast cancer has been shown to inhibit the initiation and progression of tumorigenesis (Guo et al., 2006; Lahlou et al., 2007; White et al., 2004).

The progression of breast cancer involves a distinct series of pathological steps, from benign hyperplasia to carcinoma in situ, and then to invasive carcinoma (Cardiff et al., 2000). Invasive carcinoma pathology is denoted by the breach of the basal membrane that normally supports the mammary epithelium and prevents the carcinoma from metastasizing through the lymphatic or blood circulation. Similar to other types of cancers (including leukemia, melanoma and sarcoma), carcinomas are highly heterogeneous (Heppner and Miller, 1983). Hence, a neoplastic lesion comprises several populations of cells that display various levels of differentiation. Presently, two major models of how this heterogeneity is generated within a tumor prevail – a 'cancer stem cell' model and a 'clonal evolution' model (Campbell and Polyak, 2007) (Fig. 1). In the cancer stem cell model, random mutations that are accumulated by normal stem cells or their early progeny throughout the life of an individual are proposed to lead to the generation of proliferating cancer stem cells. These cells would undergo an altered version of the normal differentiation process, and would drive tumor progression and its recurrence (Reya et al., 2001). By contrast, the clonal evolution model is largely based on the hypothesis that any undifferentiated or differentiated cell can accumulate mutations that lead to tumor formation and to the generation of clonal populations of cells within the tumor. This clonal evolution model also predicts the generation within tumors of 'cancer stem cells' – mutated cancer cells that are devoid of terminal differentiation markers and display stem-cell-like properties, an idea that is supported by the demonstration that differentiated cells can acquire stemness following the overexpression of the appropriate panel of transcription factors (Hochedlinger and Jaenisch, 2006). Whatever the mechanism that leads to the production of cancer stem cells, an understanding of their biology is crucial given the high mortality rate that is associated with the presence of undifferentiated cancer cells within tumors. Breast tumors that are enriched in undifferentiated cancer cells are typically more aggressive and prone to metastasis, resistance to therapy and relapse events, which can occur either at the primary tumor site or at distant metastatic sites following an apparent remission or dormancy for several years (Brackstone et al., 2007).

Thus, the therapeutic targeting of cancer stem cells promises to be clinically fruitful.

Multiple parallels have been drawn between stem cells and cancer stem cells, which should help us to understand the innate biology of cancer stem cells. Although the relative quiescence of normal stem cells contrasts with the uncontrolled proliferation capacity of cancer stem cells, the two cell types do share several essential properties – they have the ability to self-renew, they express high levels of ABC-type transporters that confer resistance to drug treatments (Dean et al., 2005), they exhibit a relative resistance to radiation (Bao et al., 2006), and they are mobile and can survive for long periods of time without any anchorage (Reya et al., 2001). β1 integrin has been shown to be highly expressed in normal stem cells and to regulate their biology in various organs – the hematopoietic system (Laird et al., 2008), the brain (Campos, 2005) and epithelial organs such as the skin (Watt, 2002) and more recently the mammary gland (Shackleton et al., 2006; Stingl et al., 2006). In the mammary gland, β1 integrin has been proposed to directly regulate the ability of mammary stem cells to self-renew and differentiate properly (Taddei et al., 2008). In this Commentary, we propose that these findings reflect a role for integrins in the biology of mammary-cancer stem cells, and should be informative about the actual role of integrins during the initial steps of breast-cancer progression.

Following a presentation of the recent advances that have been made concerning the role of integrins in mammary stem cells, we will briefly present experimental data that support a role for integrins during the initiation of tumorigenesis in vivo and will analyze, in light of our current knowledge of mammary-cancer stem cells, how these results suggest that integrins regulate the biology of cancer stem cells.

Integrins in mammary stem cells and their differentiation

The mammary gland

The mammary gland undergoes essential steps in its development after birth. It comprises an epithelium with two cell layers – a basal population that contains stem cells and myoepithelial cells that rest on a basement membrane, and a differentiated luminal population that stands above the basal population (Chepko and Smith, 1999) (Fig. 2). Basal and luminal epithelial cells form a complex alveolo-tubular network that is embedded in a specialized underlying stroma, the mammary fat pad. At puberty, ducts develop through dichotomous branching and extend to the edge of the fat pad. Additional differentiated structures called alveoli sprout all over the ductal network and differentiate in order to produce milk to feed offspring during pregnancy and lactation. When lactation ends, the mammary-gland epithelium regresses until the next pregnancy (Brisken and Rajaram, 2006).

Expression and localization of integrins in the mammary-gland epithelium and their role in mammary-gland development

Similar to their expression in the skin (Watt, 2002), integrins are more highly expressed in the basal cell layer of the mammary epithelium than in its differentiated luminal compartment. Only few integrin heterodimers have been shown to be significantly expressed (Taddei et al., 2003) in the mammary epithelium, all of which are β1-integrin- and β4-integrin-containing heterodimers (Fig. 2). Despite their similar expression pattern, β1-integrin- and β4-integrin-containing heterodimers are implicated in very distinct adhesion complexes. The α6β4 heterodimer connects myoepithelial cells to the underlying basement membrane by forming hemidesmosome structures that link cytosolic keratins to extracellular laminin (Litjens et al., 2006). The localization of β1-integrin-containing heterodimers is more complex. They can localize basally in membrane domains, where they oligomerize into focal-adhesion structures to form a connection between extracellular components, lipids, and cytoskeletal and signaling components of the cytosol; in addition, they localize laterally at cell-cell junctions of epithelial cells (Naylor et al., 2005; Taddei et al., 2003).

In contrast to skin, in which a blistering phenotype was observed following the deletion of β4 integrin (Dowling et al., 1996), the mammary epithelium seems to develop normally when α6 integrin is deleted or when β4 integrin is inhibited (Klinowska et al., 2001; Nikolopoulos et al., 2004). The overexpression of a dominant-negative mutant of β1 integrin (Faraldo et al., 1998), or the conditional deletion of β1 integrin using targeted Cre recombinases in either the basal population (Li et al., 2005; Naylor et al., 2005; Taddei et al., 2008) or in terminally differentiating luminal cells during pregnancy and/or lactation (Li et al., 2005; Naylor et al., 2005), perturbs both ductal outgrowth and alveologenesis.

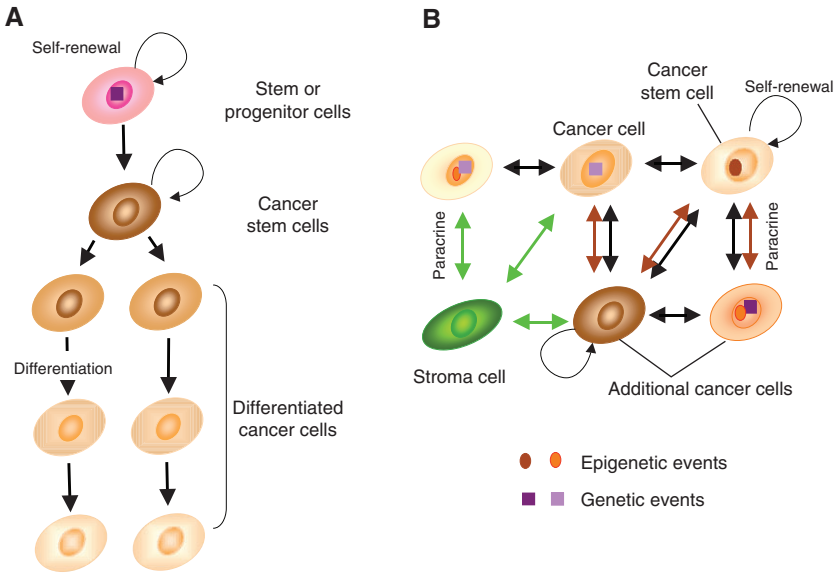

Fig. 1. The two main working models describing the generation of tumor heterogeneity. (A) The 'cancer stem cell' model. Accumulation of genetic mutations in stem cells might lead to the formation of cancer cells with self-renewal properties (cancer stem cells). These cancer stem cells drive tumor progression and heterogeneity by proliferating and generating some differentiated cancer cells. (B) The 'clonal evolution' model. In this model, epigenetic and genetic events can induce the transformation, dedifferentiation and acquisition of self-renewal properties in any cell type. The evolution of cancer cells that derive from the original cell is unstable, and depends on the surrounding environment and on paracrine signals that come either from stroma cells (green arrows) or from other tumor cells (brown arrows). The two models are not necessarily mutually exclusive.

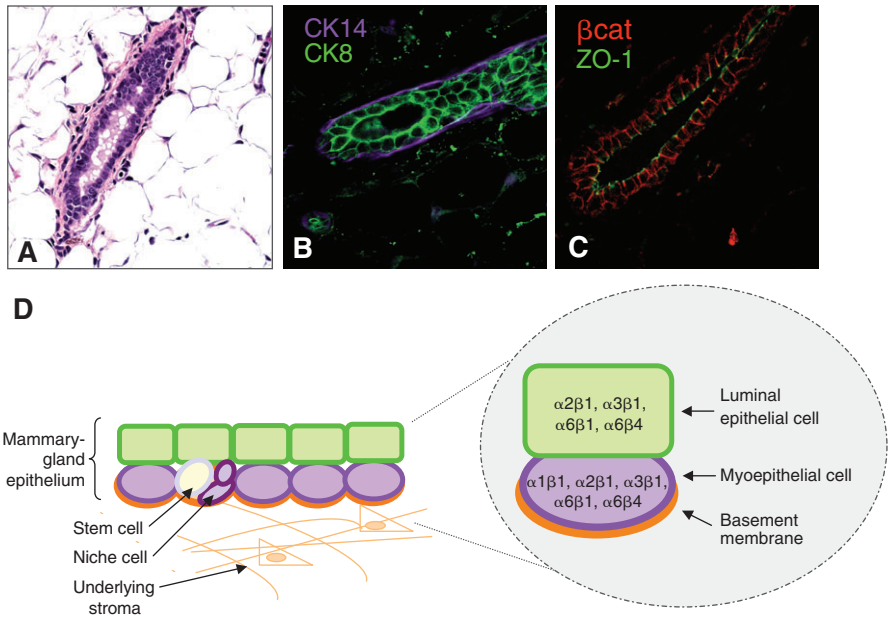

Fig. 2. The structure of the mammary epithelium. (A) Paraffin sections from a 10-week-old mouse mammary gland stained with hematoxylin-phloxin-safran dye. The surrounding fat pad is visible. (B) Paraffin section labelled with markers of epithelial luminal cells (anti-cytokeratin 8; green) and basal cells (anti-cytokeratin 14; purple). (C) Polarization of the luminal epithelium is illustrated by labeling for polarity markers zonula occludens 1 (ZO-1; apical, green) and β-catenin (βcat; basolateral, red). (D) Schematic representation of the structure of the mammary epithelium and the different integrin heterodimers expressed in luminal epithelial cells and myoepithelial cells.

Integrin expression in mammary stem and progenitor cells

Consistent with its role in mammary gland ductal outgrowth (Taddei et al., 2008), β1 integrin has been recently used as a marker to purify mammary stem cells (Shackleton et al., 2006; Stingl et al., 2006). Indeed, cells that express high levels of both α6 and β1 integrins (also known as CD49f and CD29, respectively), as well as low levels of the epithelial marker CD24, were able to regenerate an entire mammary gland. Cells presenting a $CD24^{low}CD49f^{high}$ or $CD24^{low}CD29^{high}$ molecular signature have therefore been characterized as mammary stem cells (Shackleton et al., 2006; Stingl et al., 2006). In contrast to their differentiated progeny, mammary stem cells are negative for estrogen receptor (ERα), progesterone receptor (PR) and the tyrosine kinase receptor HER2 – three molecular markers that define different populations of differentiated luminal epithelial cells – but are highly positive for the transcription factor p63, the epidermal growth factor receptor (EGFR) and cytokeratin 14 (CK14), confirming their basal origin (Asselin-Labat et al., 2006).

In addition to mammary stem cells, a transit-amplifying luminal committed stem-cell-progeny population (progenitor cells) has also been shown to express high levels of another integrin, β3 integrin (also known as CD61), which is only marginally expressed in normal mammary epithelia (Taddei et al., 2003). Indeed, the inhibition of luminal epithelial-cell differentiation that results from the deletion of the GATA3 transcription factor (Asselin-Labat et al., 2007; Kouros-Mehr et al., 2006) has been shown to lead to the accumulation of mammary progenitor cells that are characterized by their high CD61 expression and their low expression of α6β1 integrin (Asselin-Labat et al., 2007). The $CD24^{high}CD29^{low}CD61^{high}$ molecular signature is believed to be common to two distinct populations of luminal progenitors, one that gives rise to the luminal cells of the mammary ducts and one that gives rise to the luminal cells of the alveolar structure (Stingl and Caldas, 2007). These two populations can be differentiated by the level of expression of stem cell antigen 1 (Sca1, also known as LY6A), which is highly expressed in ductal luminal progenitors (Stingl and Caldas, 2007).

Although the binding of β3 integrin to interstitial matrix components, such as vitronectin, osteopontin or fibronectin (van der Flier and Sonnenberg, 2001), might support the migration of progenitor cells during the differentiation process, its actual molecular role in these cells remains to be determined. By contrast, some of the signaling functions of β1 integrin in the biology of mammary stem cells and their progeny have started to be unveiled in several recent publications. Taddei et al. have shown that β1 integrin is essential for the regenerative potential of the adult mammary gland (Taddei et al., 2008), whereas other publications have demonstrated its essential role in major morphogenesis events that occur in the mammary gland during pregnancy and lactation (Li et al., 2005; Naylor et al., 2005).

β1 integrin signaling and the maintenance of the pool of adult mammary stem cells

As has been mentioned above, mammary stem cells are localized in the basal layer of the adult mammary-gland epithelium, where they rest on the basement membrane (Fig. 2D; Fig. 3A). In combination with some specialized basal cells that remain to be fully characterized, it is believed that the basal membrane constitutes the stem-cell niche (Brisken and Duss, 2007), which regulates when and how stem cells proliferate and differentiate (Watt and Hogan, 2000). Recently, integrin-mediated adhesion has been reported to be essential for the correct positioning of the niche around the stem-cell population in *Drosophila*, and loss of βPS integrin subunit results in depletion of the stem-cell pool (Tanentzapf et al., 2007). In mouse, deletion of the gene encoding β1 integrin in mammary basal cells is associated with depletion of the $CD29^{high}CD24^{low}$ mammary-stem-cell population and abrogates the regenerative potential of the adult mammary gland (Taddei et al., 2008). Hence, these results suggest that β1-integrin deletion either perturbs the structure of the stem-cell niche in the adult mouse mammary gland (as has been observed in *Drosophila*; see above) and/or alters the ability of stem cells to conserve their stemness and remain in the epithelium.

Interestingly, a dramatic perturbation of the orientation of the mitotic spindle in dividing basal cells also occurs during regeneration of β1-integrin-deleted glands (Taddei et al., 2008). Instead of dividing symmetrically as control cells do, β1-integrin-deleted basal cells divide both symmetrically and asymmetrically; asymmetric division gives rise to daughter cells that express molecular markers of the luminal lineage (Taddei et al., 2008). Depending on the developmental phase, stem cells need to be either amplified or merely maintained, and they therefore need to divide symmetrically or asymmetrically to give rise to either two stem

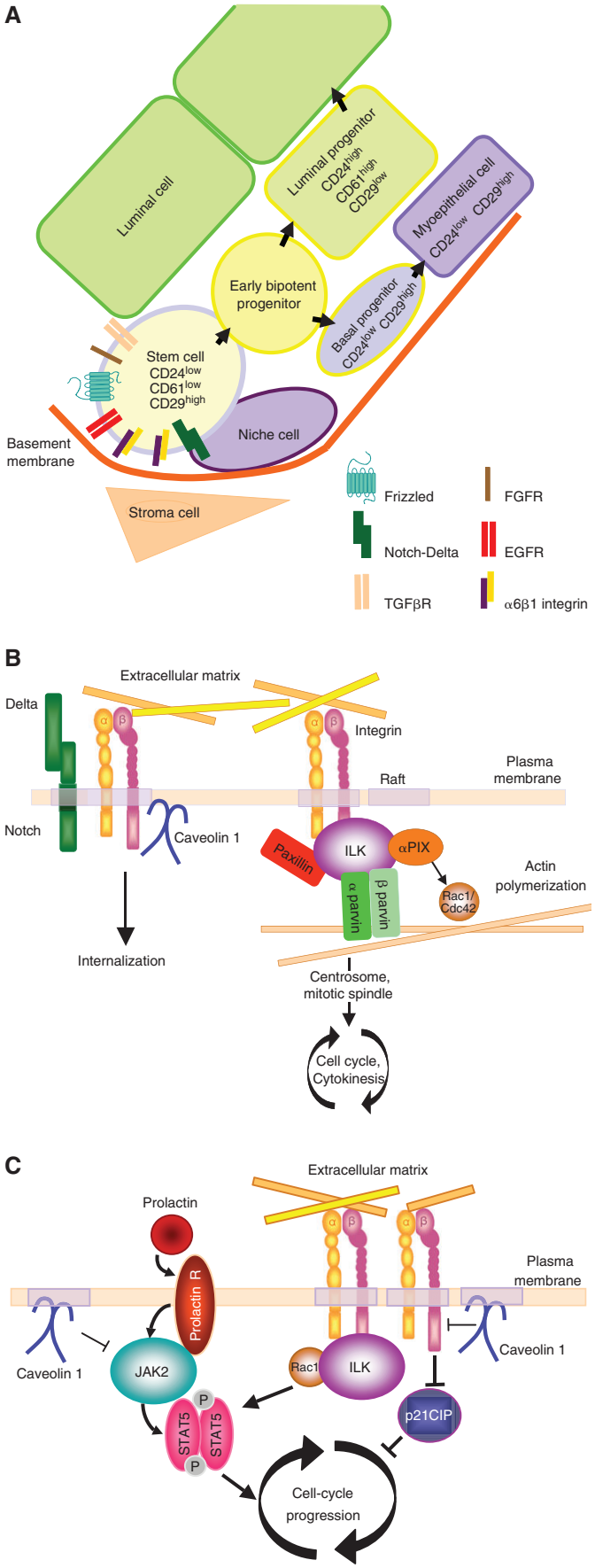

Fig. 3. Integrin expression and signaling in mammary stem and progenitor cells. (A) Representation of the adult mammary-stem-cell niche and the surface molecular markers expressed by stem- and progenitor-cells. Membrane receptors that have been implicated in mammary-stem-cell biology and the regulation of the stem-cell pool are also represented, including Frizzled, the epidermal and fibroblast growth factor receptors (EGFR and FGFR), Notch (with its ligand Delta present at the surface of adjacent niche cells) and the transforming growth factor β receptor (TGFβR). (B) The signaling role of β1 integrin in stem cells. β1 integrin (probably in association with α6 integrin) ensures the adhesion of mammary stem cells to the surrounding extracellular matrix of the niche, probably through the activation of the adhesion pathways that are classically associated with integrins (not represented here). In addition, β1 integrin influences the formation and orientation of the mitotic spindle during cell division. β1-integrin partners (such as ILK and paxillin) have been also implicated in this event. ILK is involved in two different types of protein complexes – one at focal adhesions, where ILK interacts with paxillin, α- and β-parvin, αPIX and Rac1 and regulates actin polymerization (Fielding et al., 2008a), and another at the centrosome (not represented). Although β1 integrin action might be dependent on ILK, it is currently unclear whether β1 integrin regulates one or both of these ILK pools. An independent (and speculative) interaction between the Notch receptor and β1 integrin is also represented – β1 integrin might modulate Notch activity by promoting its internalization through a caveolin-1-dependent process. (C) β1-integrin signaling during alveologenesis and alveolar differentiation. During pregnancy, prolactin begins to be produced and induces the amplification of alveolar progenitors, as well as their differentiation. Binding of prolactin to its receptor (Prolactin R) induces the activation of Janus kinase 2 (JAK2) and the subsequent phosphorylation of STAT5. In this event, β1 integrin appears to be essential to mediate the correct phosphorylation of STAT5. ILK and Rac1 are probably involved in this β1-integrin-associated signaling. Caveolin 1, which is associated with lipid-raft membrane domains, has been shown to downregulate both JAK2 and β1-integrin activity. In addition to promoting proliferation through STAT5, β1 integrin promotes cell-cycle progression by inducing the proteasomal degradation of the cyclin inhibitor p21CIP.

cells, or to one stem cell and a progeny cell that will undergo subsequent differentiation (Smith and Medina, 2008).

Both in *Drosophila* (Fernandez-Minan et al., 2007) and in mammals (Lechler and Fuchs, 2005), β1 integrin appears to ensure the correct orientation of the mitotic furrow during stem-cell division. In vitro, β1 integrin has been shown to regulate the very early steps of furrow formation by modulating microtubule growth from centrosomes and assembly of the mitotic spindle (Reverte et al., 2006). Although the β1-integrin-coupled signaling molecules involved in this process remain to be elucidated in the mouse mammary gland (Fig. 3B), several cytoplasmic integrin-associated proteins, such as paxillin, HEF1, zyxin and integrin-linked kinase (ILK), have been shown to localize to centrosomes and to regulate aspects of mitotic-spindle function (Fielding et al., 2008a). ILK, which binds to integrin cytoplasmic domains and is a major regulator of integrin-mediated actin-cytoskeleton organization, also regulates the assembly of the mitotic spindle, and its inhibition results in mono-astral spindles (Fielding et al., 2008b). Studies in *Drosophila* have also demonstrated a role for ILK in mitosis (Bettencourt-Dias et al., 2004).

β1 integrin promotes proliferation and differentiation

At mid-pregnancy, the hormone prolactin starts to be produced and mobilizes the alveolar progenitor population, promoting the formation of alveolar structures all over the mammary-gland epithelium (Brisken and Rajaram, 2006). Around parturition, alveoli differentiate and start to produce milk. The role of β1 integrin in these terminal morphogenesis events has been studied in detail. Conditional deletion of β1 integrin using Cre recombinase under the control of the promoter of either β-lactoglobulin (Blg-Cre) or whey acidic protein (WAP-Cre), which are expressed from 12 weeks of

age in nulliparous mice or at mid-pregnancy, respectively, has been used to study the role of β1 integrin at different stages of differentiation of the epithelium during pregnancy and lactation (Li et al., 2005; Naylor et al., 2005). Deletion of β1 integrin using Blg-Cre or WAP-Cre induced a major defect in lactogenesis (Li et al., 2005; Naylor et al., 2005). Alveolar density in β1-integrin-null mammary glands at mid-pregnancy or at day 2 of lactation was dramatically decreased compared with control glands, and alveolar structures were impaired (Li et al., 2005; Naylor et al., 2005). Signaling analysis (Fig. 3C) showed that β1-null alveolar cells were unable to respond to the presence of prolactin and to phosphorylate the STAT5 transcription factor (Naylor et al., 2005), which has been shown to be essential for mediating prolactin-induced proliferation (Hennighausen and Robinson, 2005). ILK appears to mediate the β1-integrin-dependent activation of STAT5 by acting upstream of Rac1 (Akhtar and Streuli, 2006). In addition to inactive forms of STAT5, β1-integrin-null epithelial cells also displayed a significant increase in the level of the cyclin inhibitor p21CIP. Importantly, co-deletion of p21CIP and β1 integrin partially reverts the observed lack of proliferation of the epithelium during lactation (Li et al., 2005). These results are consistent with previous findings showing that β1-integrin promotes proliferation by inducing the proteasomal degradation of p21CIP and p27KIP (an additional cyclin inhibitor) (Giancotti and Tarone, 2003).

Overall, these results suggest that β1 integrin is essential both for the mobilization of alveolar progenitors and the initiation of the alveologenesis process, but also at a later stage when alveoli differentiate in order to produce milk. The importance of β1 integrin in this later stage of alveolar differentiation is probably associated with the capacity of β1 integrin to mediate adhesion of alveolar cells to the surrounding ECM, suggesting that classic integrin-associated adhesion pathways are at work during this process. However, the role of integrin in the initiation of the proliferation of alveolar progenitors seems to be related to its ability to mediate STAT5 activation and positively influence mitosis. Whether its role is restricted to activation of STAT5 and repression of p21CIP expression or is also related to the regulation of cytokinesis remains to be determined. In addition to these direct effects of β1 integrin on proliferation, other mechanisms that lead to activation of integrin signaling might also be at work during the proliferation stages of alveologenesis. Indeed, prolactin signaling has been shown to inhibit the expression of caveolin 1, an important regulator of β1-integrin signaling (Park et al., 2001). Caveolin 1 is implicated in the dynamic regulation of the presence at the plasma membrane of cholesterol-enriched nanodomains (lipid rafts) (Lajoie and Nabi, 2007), and negatively regulates β1 integrin by promoting its internalization and its removal from the plasma membrane (del Pozo et al., 2005). Interestingly, the deletion of caveolin 1, which promotes amplification of both mammary stem cells and progenitor cells in the mammary gland (Sotgia et al., 2005), induces precocious phosphorylation of STAT5 and the process of alveologenesis (Sotgia et al., 2006).

The signaling activity of β1 integrin appears to be essential for determining the lineage specification and differentiation of mammary stem cells. In the mammary gland, a number of receptors (Fig. 3A), such as Frizzled (the receptor of the Wnt1 ligand), the epidermal and the fibroblast growth factor receptors (EGFR and FGFR), the transforming growth factor β receptor (TGFβR) and the Notch receptor, are known to participate in the biology of stem cells by regulating their proliferation and differentiation (Brisken and Duss, 2007). However, very few data exist regarding lineage specification during differentiation. The Notch receptor has recently been implicated in the specification of luminal cell fate of mammary stem cells (Bouras et al., 2008). Interestingly, in neural stem cells, a functional and physical interaction between β1 integrin and the Notch receptor has been described (Campos, 2005). In those cells, β1 integrin regulates and tunes Notch-receptor activation by mediating its partial internalization via a caveolin-1-dependent mechanism (Campos, 2005). These results suggest that β1 integrin might modulate Notch activity during the generation of luminal progenitors in the mammary gland. It will be interesting to determine whether this putative Notch-integrin functional interaction influences the generation of both alveolar and ductal luminal progenitors in a similar way.

In addition to its apparent role in the maintenance and the differentiation of mammary stem cells, β1 integrin and its signaling partners have also recently been shown to participate in the very early events of neoplastic development. These data are discussed below.

Integrins and their role in the initiation and progression of breast cancer

Although the role of integrins in the proliferation and migration of cancer cells, including breast-cancer cells, has been extensively reviewed and is supported by a substantial number of publications (Giancotti and Tarone, 2003; Guo and Giancotti, 2004; White and Muller, 2007), it is only recently that integrins have been directly implicated in the early events of tumorigenesis in vivo. Data clearly demonstrate that both β1 and β4 integrin (the two β-subunits that are expressed in the normal mammary gland) (Taddei et al., 2003), as well as some of their signaling partners [such as the focal-adhesion kinase (FAK)], are important for tumor progression.

In a breast-cancer mouse model, in which an activated version of the human oncogene *ERBB2* (also known as HER-2 and NEU) is expressed under the control of a mammary-epithelium-specific promoter (Ursini-Siegel et al., 2007), the expression of a dominant-negative mutant of β4 integrin has been shown to delay ERBB2-induced tumorigenesis significantly and to suppress metastasis (Guo et al., 2006). β4 integrin, which has been shown to interact directly with the ErbB2 receptor, appears to be essential both for proliferation and survival, and for resistance to immunotherapy, through the activation of the Jun and STAT3 pathways, respectively (Guo et al., 2006). Interestingly, other signaling partners of β4 integrin, including p63 (Carroll et al., 2006) and NFκB (Weaver et al., 2002), have also been independently demonstrated to be crucial for cell survival and resistance to apoptosis. p63, which is normally expressed specifically in basal cells (either normal or cancerous mammary basal epithelial cells or stem cells) can induce β4-integrin expression to mediate resistance to anoikis (an apoptotic mechanism that is induced by loss of cell anchorage) through a STAT3-dependent mechanism (Carroll et al., 2006). NF-κB has also been implicated in the signaling mechanisms that promote β4-integrin-mediated resistance to apoptosis (Weaver et al., 2002). Taken together, these data strongly argue that targeting β4 integrin might become a relevant strategy in the treatment of breast cancer to restrict metastasis and cancer-cell survival.

In the ERBB2 mouse model, β4 integrin appears only to diminish cancer initiation, but the mammary-gland-specific disruption of β1 integrin has been shown to essentially block the tumorigenesis that is associated with another oncogene, the polyomavirus middle T antigen (PyMT) (White et al., 2004). Similar to previous observations in vitro (Weaver et al., 1997), β1-integrin ablation in vivo results in an inhibition of the activity of integrin-coupled signaling molecules such as FAK (White et al., 2004). Consistent with the importance of

this downstream β1-integrin signaling pathway, mammary-gland-specific deletion of FAK has also recently been shown to have a significant negative impact on PyMT-associated tumor progression (Lahlou et al., 2007). Unlike the complete inhibition of tumorigenesis that is observed in mice lacking β1-integrin function, pre-neoplastic lesions can still be detected when FAK expression is disrupted (Lahlou et al., 2007). Similar to β1-integrin-deficient epithelia (White et al., 2004), however, these lesions are unable to proliferate. These analyses reveal that FAK is required for the progression towards invasive carcinoma, but they also suggest that β1 integrin, the upstream regulator of FAK, has additional roles in tumor initiation, given the more drastic phenotype observed when this integrin is deleted in the PyMT mouse model [transgenic mice that express the polyomavirus middle T (PyV-mT) gene under the MMTV promoter] (White et al., 2004). Whether the β1-integrin pathway has similar importance in other types of mouse cancer models remains to be definitively established. However, these seminal studies strongly support the notion that β1 integrin is implicated in the very early steps of tumorigenesis.

As has been mentioned in the Introduction, a number of researchers propose that cancer stem cells constitute the driving force of tumorigenesis. Taken together, data that highlight the role of β1 integrin in normal mammary stem cells and in the initiation of tumorigenesis suggest that β1 integrin regulates the generation and the amplification of cancer stem cells during cancer progression. A possible role for integrins in mammary-cancer stem cells is discussed in more detail in the following section.

Mammary-cancer stem cells – a role for integrins?

Isolation of mammary-cancer stem cells

Several groups have isolated and characterized putative human mammary-cancer stem cells, using a number of markers including the epithelial marker CD24 and the hyaluronic-acid receptor CD44 (Al Hajj et al., 2003; Shipitsin et al., 2007). The results of these analyses have shown that cancer cells that express low levels of CD24 and high levels of CD44 exhibit cancer-stem-cell behavior and a specific molecular signature (Shipitsin et al., 2007). Interestingly, the precision of cancer-stem-cell isolation has been recently improved by the use of an additional marker of purification, aldehyde dehydrogenase (ALDH), which – in association with the CD44 marker – should allow the isolation of cancer stem cells with a higher degree of purity (Ginestier et al., 2007).

To date, studies on the cellular characteristics of mammary-cancer stem cells that express high levels of CD44 have shown that they display strong invasive properties (Al Hajj et al., 2003; Sheridan et al., 2006). These increased metastatic properties of CD44-expressing cancer cells might be related to the very diverse signaling abilities of this protein (Ponta et al., 2003). Indeed, CD44 has been shown to promote the activity of metalloproteases such as MMP2 and MMP9 (Ponta et al., 2003), but expression of CD44 also correlates with the expression of proteins that are implicated in the epithelial-mesenchymal transition (Mani et al., 2008), which is thought to have a role in metastasis (Turley et al., 2008). Alternatively, the direct binding of CD44 to adhesion molecules that are present in the vascular endothelium might promote trans-endothelial migration (Zen et al., 2008) and, therefore, invasion.

In humans, a single population of cancer stem cells, which is defined by its ALDH1^{+}CD44^{high}CD24^{low} expression signature, has been isolated so far (Al Hajj et al., 2003; Ginestier et al., 2007); however, mouse models of breast cancer have revealed a much more complex situation. Although the use of mice that overexpress ERBB2 or in which the tumor suppressor breast cancer 1 gene (*Brca1*) is deleted, has allowed the purification of cancer stem cells that express ALDH1 (Korkaya et al., 2008) or the CD44 and CD24 markers (Wright et al., 2008), respectively, other membrane-surface markers have allowed the purification of cancer stem cells that are distinct from the ALDH1^{+}CD44^{high}CD24^{low} cancer-stem-cell population. Indeed, *Brca1*-deleted mammary-gland-derived tumors have been shown to contain a second cancer-stem-cell population that is defined by the expression of the marker prominin 1 (also known as CD133) (Wright et al., 2008). The idea that CD44 is not a universal marker of cancer stem cells has been confirmed in another mouse model, in which the gene encoding the tumor suppressor p53 is deleted (Zhang et al., 2008). Using β1 integrin (CD29) and CD24 to purify cancer stem cells, these authors clearly demonstrate that CD29^{high}CD24^{low} cancer stem cells behave similarly whatever the level of their CD44 expression (Zhang et al., 2008).

Overall, these recent results obtained in breast-cancer mouse models strongly suggest that CD44 is not a universal marker for cancer stem cells, and demonstrate that several populations of cancer stem cells might coexist together in a single type of tumor. However, few studies have so far tackled how cancer stem cells relate to normal mammary stem cells. The results obtained independently by Ginestier et al. and Zhang et al. constitute the first experimental evidence that at least some mammary-cancer stem cells can be isolated using the same molecular markers as are used for normal mammary stem cells, in both human and mouse (Ginestier et al., 2007; Zhang et al., 2008). The next question is whether these cancer stem cells drive cancer progression by undergoing an uncontrolled version of normal differentiation, as is proposed in the cancer-stem-cell model.

How do cancer stem cells relate to normal mammary stem cells?

Studies performed in mice overexpressing Wnt1, the ligand of the Frizzled receptor, under the mouse mammary tumour virus (MMTV) promoter (Wnt1 mice), are particularly helpful in determining the relationship between normal mammary stem cells and cancer stem cells. In the mammary gland, overexpression of Wnt1 has been shown to promote amplification of the mammary-stem-cell compartment (Shackleton et al., 2006) and leads to tumor formation (Li et al., 2003). As in the p53 mouse model (see above), cancer stem cells can be purified from tumors of Wnt1-overexpressing mice by using the CD29^{high}CD24^{low} signature (Vaillant et al., 2008). Wnt1-induced tumor progression correlates with the amplification of a population of progenitor cells that is characterized by the expression of Sca1 and cytokeratin 6 (SwissProt reference number: P48666); these represent almost half of the epithelial cells observed in hyperplastic mammary gland and in tumors (Li et al., 2003). Further studies will, however, be necessary to determine whether Sca1^{+} cells are actually a differentiated variant of CD49^{high}CD24^{low} cancer stem cells. More recently, a minor population of cancer cells that is characterized by the CD24^{high}CD29^{low}CD61^{high} signature, which is specific for mammary luminal progenitor cells (Asselin-Labat et al., 2007), has also been detected in Wnt1 tumors (Vaillant et al., 2008). In contrast to normal mammary luminal progenitors, which do not exhibit any regenerative properties, this population of cancer cells displays cancer-stem-cell properties and a high tumorigenic potential in both Wnt1- and p53$^{-/-}$-mouse-derived tumors (Vaillant et al., 2008). This population has been shown to be amplified to a particularly large extent in mice overexpressing the oncogenic fusion protein

ETV6-NTRK3 (Li et al., 2007) or the *PyMT* oncogene (Kouros-Mehr et al., 2008). In PyMT-induced carcinoma, cells that display the $CD24^{high}CD29^{low}CD61^{high}$ signature represent 80% of the tumor, consistent with the extreme aggressiveness of this oncogene. The amplification of one particular population of cancer progenitor cells with putative cancer-stem-cell properties, as observed in the PyMT model, contrasts with data obtained from Wnt1 mice, in which several types of cancer cell with mammary-stem-cell or mammary-stem-cell-progenitor signatures appear to participate in tumor progression (Kouros-Mehr et al., 2008; Vaillant et al., 2008). These results are consistent with the high histological heterogeneity that is detected in tumors derived from Wnt1 mice (Vaillant et al., 2008).

Altogether, the results described above are consistent with the idea that cancer stem cells are related to normal stem cells in their ability to give rise to cancer progenitor cells and to differentiate; however, it is still not established whether mammary-cancer stem and progenitor cells always derive from mammary stem cells. As described in the Introduction, several models might be at work to support the generation of cancer stem cells and cancer progenitor cells (Fig. 1). Although the 'cancer stem cell' model is likely to describe cancer progression in mouse models such as Wnt1 or BRCA1 (Vaillant et al., 2008), it is still unclear whether some more-homogeneous tumors such as those found in ERBB2 or PyMT mouse models might be more appropriately described by the 'clonal evolution' model (Fig. 1). Alternatively, these powerful oncogenes might also strongly control the lineage specification of mammary stem cells, inducing the formation of a single population of $CD24^{high}CD29^{low}CD61^{high}$ cancer progenitor cells that displays a very high tumorigenic (Vaillant et al., 2008) and metastatic potential (Kouros-Mehr et al., 2008).

A role for integrins in cancer-stem-cell biology?

The fact that some cancer stem cells appear to relate to normal mammary stem cells in their ability to self-renew and give rise to several populations of cancer cells suggests that similar fundamental signaling pathways are at work in these two types of stem cells. As has been described above for mammary stem cells, integrins – and especially β1 integrin – have been implicated in the self-renewal properties of stem cells (Lechler and Fuchs, 2005; Taddei et al., 2008), as well as in their differentiation (Naylor et al., 2005; Watt, 2002). An obvious hypothesis is that integrins constitute key molecular factors in cancer-stem-cell self-renewal and regulate their differentiation pattern. Interestingly, results obtained in PyMT mice suggest that β1 integrin is required for either the generation or the amplification of $CD24^{high}CD29^{low}CD61^{high}$ cancer cells (White et al., 2004; Kouros-Mehr et al., 2008). Given the drastic block of tumorigenesis that is observed following the deletion of β1 integrin (White et al., 2004), it is possible that β1 integrin is active very early in the formation of this population – in contrast to FAK (Lahlou et al., 2007), which might be involved in the amplification of the population. Hence, it will be interesting to determine the identity of the β1-integrin heterodimers that are implicated in these different stages of PyMT-induced tumorigenesis.

Conclusions and perspectives

The recent findings that integrins such as β1 integrin participate in the regulation of stem-cell biology and are required for cancer progression seem to indicate their importance in the processes that drive the generation and maintenance of cancer stem cells. To test this hypothesis, it will be important to evaluate how loss of function of these integrins affects the amplification of cancer stem cells or progenitor cells that is observed in the various breast-cancer mouse models that are currently available. Given the large panel of integrin heterodimers that might be expressed in cancer stem cells ($CD24^{low}CD29^{high}CD61^{low}$) or cancer progenitor cells ($CD24^{high}CD29^{low}CD61^{high}$), it will be particularly important to determine in the future which of those heterodimer(s) are implicated in either the self-renewal or differentiation of stem cells or cancer stem cells. With that knowledge, targeted activation or inhibition of specific integrin-signaling pathways might constitute some powerful strategies to impair the self-renewal potential of cancer stem cells or modulate the oncogene-driven differentiation of mammary-gland cells. Such advances in our ability to modulate the biology and differentiation potential of cancer stem cells will hopefully allow the development of novel therapies to increase breast-cancer survival and, eventually, eradicate this debilitating disease.

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