faces of the vaginal walls. With biweekly infusions, the levels of the neutralizing antibody, called b12, were substantially lower than those needed to protect from high, intravenous doses of SIV in previous experiments. A larger number of low-dose vaginal challenges was needed to infect the macaques infused with b12 compared to macaques that had not received b12; a similar study from this group also showed protection with another neutralizing antibody, called 2G12 (ref. 6).

Infusion with the b12 antibody delayed viral infection, and there was a trend toward lower viremia in those macaques that became infected[4], an indication of better clinical outcomes in humans and macaques. Hessell *et al.* further showed that a mutant of b12 that cannot interact with antibody receptors did not provide protection from infection.

In a complementary study, Johnson *et al.*[5] expressed an engineered neutralizing antibody for SIV (distinct from b12 and 2G12) *in vivo* using a gene-transfer system with the adeno-associated virus (AAV). The AAV-encoded transgene produced the engineered neutralizing antibody in muscle cells. The engineered antibody was released into the blood stream and was passively distributed through the body, similar to the immune memory against antigen that is produced and maintained by B cells during an infection. The AAV strategy provides a unique long-term method of administering antibodies that does not rely on the adaptive immune system of the host. Antibodies of known or broad specificity, such as b12, could be administered as a prophylactic 'vaccine'.

Johnson *et al.* found that such expression of neutralizing antibodies provided protection from pathogenic SIV in six of the nine monkeys evaluated. This particular study used intravenous challenge at a dose known to infect 100% of challenged macaques rather than the low-dose, intravaginal challenges used by Hessell *et al.* Johnson *et al.* vaccinated monkeys with one of three AAVs that expressed various anti-SIV molecules called immunoadhesins. These immunoadhesins were engineered to bind SIV with either a functional domain from an SIV-specific antibody or the first two domains of the T cell protein CD4, which is used by SIV and HIV as a receptor. The major shortcoming of the study was that the unexpected immunogenicity of the immunoadhesins resulted in their inactivation in three of the monkeys, rendering them ineffective. Preventing anti-immunoadhesin responses is clearly an issue that must be resolved before advancing into human trials, potentially by the use of natural rather than engineered antibodies.

There are several considerable caveats to these findings. Both of these studies, and essentially all studies published to date, used viruses that were specifically matched to the neutralizing antibody tested[4,5]. As a result, it is unknown which individual antibody or cocktail of antibodies would protect an individual from any one of the diverse strains of HIV that are circulating in the population. Additionally, HIV frequently escapes from neutralizing antibodies, and no single monoclonal antibody could recognize and control all of these many variants of the virus. At present, there are too few neutralizing antibodies that could form the basis of the AAV-transferred vaccine. Finally, these are nonhuman primate studies that have yet to be validated in the clinic.

Where is the future for neutralizing antibodies as components of vaccines and potential immunotherapies? The limited capacity of HIV-positive individuals to produce neutralizing antibodies that recognize many HIV isolates have led some to conclude that further work on this strategy may not be profitable. However, the HIV antibody field is experiencing a renewed vigor, as data from more cohorts uncover people who generate antibodies against a broad range of viral isolates[7–9] and as new methods are now available for cloning neutralizing antibodies from HIV-infected humans[10,11].

These new approaches for the generation of antibodies, combined with envelope epitope mapping information, are beginning to provide glimpses into the antibody repertoire in subjects with broadly reactive neutralizing antibodies[12]. Together, this information may, on the one hand, aid the development of effective immunogens and, on the other, aid the development of cocktails of effective neutralizing antibodies (for passive administration or AAV-like gene therapy). Effective levels of neutralizing antibodies may be within reach.

1. Igarashi, T. *et al. Nat. Med.* **5**, 211–216 (1999).
2. Haigwood, N.L. *et al. J. Virol.* **78**, 5983–5995 (2004).
3. Nishimura, Y. *et al. J. Virol.* **76**, 2123–2130 (2002).
4. Hessell, A.J. *et al. Nat. Med.* **15**, 951–954 (2009).
5. Johnson, P.R. *et al. Nat. Med.* **15**, 901–906 (2009).
6. Hessell, A.J. *et al. PLoS Pathog.* **5**, e1000433 (2009).
7. Doria-Rose, N.A. *et al. J. Virol.* **83**, 188–199 (2009).
8. Mahalanabis, M. *et al. J. Virol.* **83**, 662–672 (2009).
9. Sather, D.N. *et al. J. Virol.* **83**, 757–769 (2009).
10. Wrammert, J. *et al. Nature* **453**, 667–671 (2008).
11. Scheid, J.F. *et al. Nature* **458**, 636–640 (2009).
12. Li, Y. *et al. J. Virol.* **83**, 1045–1059 (2009).

Mammary development meets cancer genomics

Aleix Prat & Charles M Perou

Mammary epithelial cell development is thought to progress from undifferentiated stem cells into at least two differentiated cell types. A new study has now characterized some of these distinct developmental stages and links them to tumor subtypes previously defined by gene expression profiling (pages 907–913).

Genomic studies of epithelial tumors have identified distinct subtypes with differences in survival and response to therapy, most notably for breast cancer[1]. Many researchers have speculated that these genomically defined tumor subtypes may represent transformation of stem cells with arrest at specific stages of development or, alternatively, direct transformation of various mature cell types. In this issue of *Nature Medicine*, Lim *et al.*[2] delineate the human mammary epithelial hierarchy and then use this as a framework for understanding the cellular origins of the various molecular subtypes of breast cancer. They provide a direct link between mammary development and tumor profiles.

Considerable evidence already supports an epithelial hierarchy within the human breast. This process starts with an undifferentiated estrogen receptor–negative mammary stem cell (MaSC) that maintains itself through self-renewal and differentiates into committed progenitors (whether there is one or more type of progenitor is unclear). These progenitors ultimately give rise to progeny that are the mature ductal and alveolar cells, which belong to the luminal epithelial cell lineage that line the

Aleix Prat and Charles M. Perou are at the Lineberger Comprehensive Cancer Center, Departments of Genetics and Pathology & Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA.
e-mail: cperou@med.unc.edu

lumen of the mammary gland, and the mature myoepithelial cells, which surround the luminal epithelium and contact the basement membrane. These developmental studies have been mostly based on *in vitro* differentiation assays of primary epithelial cells isolated on the basis of their expression of cell surface markers such as epithelial cell adhesion molecule (EpCAM; a general epithelial cell marker), CD49f (also known as integrin α_6) and other markers, including CD29, CD10 and aldehyde dehydrogenase-1 (ALDH-1).

At the same time, breast cancer is known to be a heterogeneous disease, and the presence of distinct molecular entities, known as the 'intrinsic' subtypes[1], suggests the existence of multiple 'cells of origin'. To link mammary development to these genomically defined tumor profiles, Lim *et al.*[2] first functionally characterized the human mammary hierarchy in normal breast specimens and then profiled them on DNA microarrays. Specifically, the authors isolated four subpopulations of breast cells by FACS analysis of EpCAM and CD49f expression. Using transplantation assays in immunocompromised mice, they showed that the $CD49f^{hi}EpCAM^{-}$ subpopulation was enriched for MaSC (bipotent) progenitors, whereas the $CD49f^{+}EpCAM^{+}$ subpopulation was enriched for 'luminal progenitors'.

Immunohistochemical analysis revealed that the MaSC subpopulation did not express estrogen receptor, progesterone receptor or keratin-8 and keratin-18 (referred to as keratin-8/18 because the antibody used recognizes both proteins) but did express the basal markers keratin-5/6 and keratin-14. In contrast, the luminal progenitor subpopulation expressed both luminal (keratin-8/18) and basal markers (keratin-5/6), which is precisely what has been described for the basal-like subtype[3]. Basal-like breast tumors are associated with poor clinical outcomes, and most lack the expression of estrogen receptor, progesterone receptor and human epidermal growth factor receptor-2 (HER2) and, thus, are clinically referred to as 'triple negative'[4]. The $CD49f^{-}EpCAM^{+}$ subpopulation expressed only luminal lineage markers including estrogen receptor and progesterone receptor, whereas the fourth subpopulation ($CD49f^{-}EpCAM^{-}$) was called 'stromal', as it did not show positivity for the epithelial lineage markers tested. This stromal subpopulation did not show enrichment for bipotent and progenitor activity despite expressing the highest levels of ALDH-1, which other researchers have shown to be a marker of MaSCs[5].

Lim *et al.* next performed gene expression profiling of each subpopulation and compared their profiles with a human data set that contained all intrinsic subtypes of breast cancer[6]. As

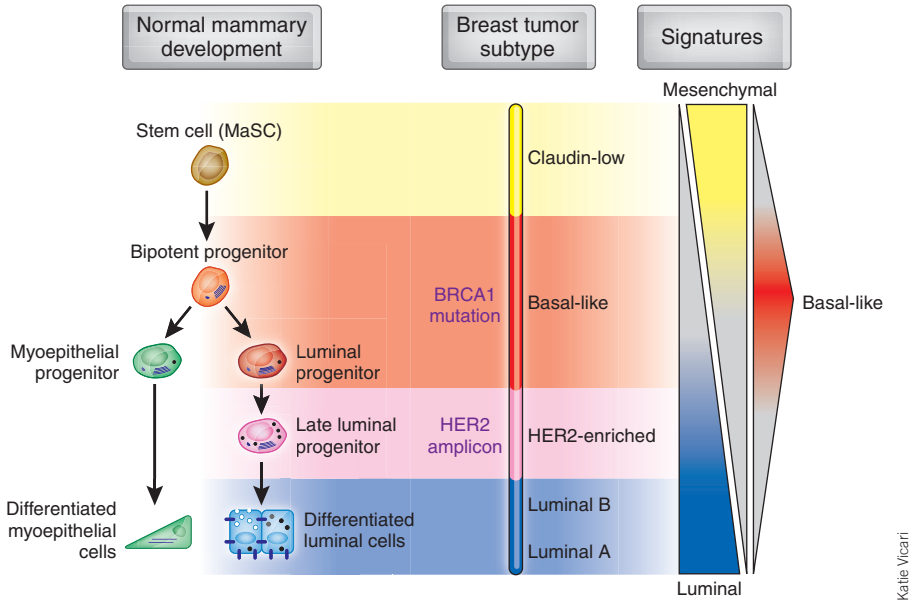

Figure 1 Model of the human mammary epithelial hierarchy linked to cancer subtype. (**a**) Subpopulations of normal breast tissue and potential cells of origin for the intrinsic subtypes of breast cancer; these cells may represent a stage of developmental arrest for a tumor with an origin earlier in the differentiation hierarchy or, alternatively, transformation of a cell type at one specific stage of development. (**b**) The various breast tumor subtypes molecularly compared to subpopulations from normal breast tissue. (**c**) The defining expression patterns of luminal, mesenchymal or claudin-low, and basal-like cells. These molecular patterns may be best represented as gradients of expression, as opposed to a discrete 'on' or 'off' state of expression. The approximate locations of the differentiation blocks imposed by BRCA1 loss and HER2 amplification are suggested by their locations in the differentiation hierarchy.

expected, the mature luminal cell signature was found to be highly enriched in the luminal A, luminal B and HER2-enriched tumors; HER2-enriched subtype tumors are primarily estrogen receptor negative, yet these and other studies show them to have luminal expression features. Conversely, the MaSC signature was found to be enriched in true normal breast tissue and in the recently identified claudin-low subtype, which is a rare subtype present in humans and mouse models that is characterized by the expression of mesenchymal and stem cell–associated genes and the lack of expression of claudin-3, claudin-4, claudin-7, E-cadherin, EpCAM and mucin-1 (refs. 6,7).

One of the most intriguing findings was that the luminal progenitor signature was found to be the most highly enriched in basal-like tumors. Previously, many investigators had speculated that basal-like tumors may represent a primitive undifferentiated cell, owing to their high-grade 'triple negativity' and poor outcomes. However, ~10–15% of basal-like tumors also express estrogen receptor[8], which is reminiscent of the infrequent but tangible staining seen for estrogen receptor in the $CD49f^{+}EpCAM^{+}$ fraction.

Our own bioinformatics analysis validates the findings of Lim *et al.*. We examined a publicly available genomic data set from Raouf *et al.*[9], who also had isolated and profiled similar human mammary epithelial subpopulations. Our analysis of their profiles resulted in findings similar to those of Lim *et al.*, namely that the basal-like tumor profile is the most similar to the 'luminal restricted progenitor' profile found by Raouf *et al.*[9] and the bipotent subpopulation is the most enriched in claudin-low tumors and normal breast specimens (A. Prat and C.M. Perou, unpublished data).

To further investigate the origin of basal-like tumorigenesis, Lim *et al.* compared the mammary epithelial subpopulations from individuals without disease to those same subpopulations from breast tissues from BRCA1 germline mutation carriers who had not developed cancer (such carriers have a lifetime risk of basal-like breast cancer of approximately 60%). Strikingly, the luminal progenitor subpopulation was found to be expanded in BRCA1 mutation carriers and showed aberrant growth properties *in vitro*. This data is concordant with previous reports suggesting a role of BRCA1 in the differentiation of stem or progenitor cells to estrogen receptor–positive mature luminal cells[10,11]. However, there are some discrepancies concerning the precise localization of the block in differentiation. For example, Liu *et al.*[11] showed that *BRCA1* gene knockdown in human mammary epithelial cells expands a potentially earlier progenitor subpopulation, that is estro-

gen receptor negative, EpCAM low and ALDH-1 high.

These new findings support the cancer stem cell (CSC) hypothesis, at least for some breast tumor intrinsic subtypes. According to this hypothesis, a cancer can arise from transformation of a normal stem cell or progenitor cell, thus giving rise to a heterogeneous population of cells. Alternatively, a differentiated cancer cell within a heterogeneous tumor may acquire stem cell–like features through acquired self-renewal mechanisms. Either way, the CSC hypothesis holds that the bulk of the tumor is composed of differentiated cells with limited proliferative potential, whereas the CSC compartment maintains the tumor and contributes to treatment resistance due to its unique biological properties[12]. Melding the CSC hypothesis with the data from Lim *et al.*[2] suggests that MaSCs and committed luminal progenitors are the cells of origin of claudin-low and basal-like tumors, respectively (**Fig. 1**). Alternatively, the MaSC may still be the cell of origin for both subtypes; however, under this scenario, claudin-low tumors may be locked in this stem cell state, whereas basal-like tumors become arrested at the luminal progenitor stage.

If basal-like tumors are arrested at a specific step in luminal development and have a minor CSC or claudin-low component, then this might explain why they have a poor prognosis despite responding to chemotherapy[13]. In this scenario, it is predicted that the residual disease present after chemotherapy treatment will be enriched for cells with stem-like features, which is precisely what has been observed[14,15]. However, isolation and better characterization of these CSCs will require further investigation. Of note, Lim *et al.*[2] isolated MaSCs on the basis of a low level of expression of EpCAM, which is currently used as the antigen to identify circulating tumor cells[16], along with keratin-8, keratin-18 and keratin-19, which are all either not expressed or expressed at low levels in MaSCs. Some researchers have suggested that circulating tumor cells may represent circulating CSCs; however, the data of Lim *et al.*[2] would argue against this.

Despite the excitement this new study is bound to elicit, investigators must keep in mind that the study of normal stem cells and CSCs has experimental limitations, including the imprecision of the markers used to purify these cells, the difficulty in performing functional reconstitution studies and the choice of functional assays. For example, the various gene expression profiles were obtained from subpopulations that were not pure cell types, a caveat that must be kept in mind when interpreting these results.

Overall, the study of Lim *et al.* improves understanding of the biological heterogeneity of breast cancer. These data suggest that an MaSC with claudin-low features progresses to a luminal progenitor state that shows characteristics of both myoepithelial (or basal) and luminal cells, which then progresses to differentiated cells with more luminal characteristics and less myoepithelial characteristics within the luminal cell lineage (**Fig. 1**). Much less is known about the myoepithelial and basal developmental lineage, which is an area that deserves more attention.

This study also further complicates the nomenclature issues surrounding basal-like breast tumors. Indeed, many in the field have questioned the use of the name 'basal-like' and the very existence of this subtype; however, these new analyses again identify the basal-like features of these tumors and, importantly, their potential normal counterparts. We support the continued use of the name basal-like, as it is still accurate, given that these tumors clearly show expression of proteins and genes that are used to define morphologically identified basal epithelial cells (that is, keratin-5, keratin-6 and keratin-14). Of note, basal-like tumors do sometimes show squamous[6] or metaplastic histology[7] and expression similarities to lung squamous carcinomas[17], thus suggesting that this differentiation cascade may also be occurring in other epithelial tumor types as well.

Irrespective of nomenclature, whether basal-like, triple negative or luminal progenitor, Lim *et al.* have advanced the field's understanding of breast tumorigenesis by providing a logical link between cancer genomics and developmental biology.

1. Perou, C.M. *et al. Nature* **406**, 747–752 (2000).
2. Lim, E. *et al. Nat. Med.* **15**, 907–913 (2009).
3. Livasy, C.A. *et al. Mod. Pathol.* **19**, 264–271 (2005).
4. Schneider, B.P. *et al. Clin. Cancer Res.* **14**, 8010–8018 (2008).
5. Ginestier, C. *et al. Cell Stem Cell* **1**, 555 (2007).
6. Herschkowitz, J.I. *et al. Genome Biol.* **8**, R76 (2007).
7. Hennessy, B.T. *et al. Cancer Res.* **69**, 4116–4124 (2009).
8. Nielsen, T.O. *et al. Clin. Cancer Res.* **10**, 5367–5374 (2004).
9. Raouf, A. *et al. Cell Stem Cell* **3**, 109–118 (2008).
10. Foulkes, W.D. *J. Med. Genet.* **41**, 1–5 (2004).
11. Liu, S. *et al. Proc. Natl. Acad. Sci. USA* **105**, 1680–1685 (2008).
12. Diehn, M. *et al. Nature* **458**, 780 (2009).
13. Carey, L.A. *et al. Clin. Cancer Res.* **13**, 2329–2334 (2007).
14. Li, X. *et al. J. Natl. Cancer Inst.* **100**, 672–679 (2008).
15. Creighton, C. *et al. Proc. Natl. Acad. Sci. USA* (in the press).
16. Cristofanilli, M. *et al. N. Engl. J. Med.* **351**, 781–791 (2004).
17. Chung, C.H., Bernard, P. & Perou, C. *Nat. Genet.* **32** Suppl, 533–540 (2002).

T time in the brain

Eng H Lo

Inflammation in neural tissue has long been suspected to have a role in stroke. A new study in mouse models of focal cerebral ischemia suggest that a stereotyped sequence of T cell infiltration and activation may underlie the progression of brain injury that can last up to days after stroke onset (pages 946–950).

Stroke remains a very challenging clinical problem. In experimental models, cerebral ischemia triggers an elevation in excitotoxic glutamate that rapidly leads to an accumulation of damaging calcium and reactive oxygen species in neurons. However, in spite of remarkable advances in the molecular biology of neuronal cell death, a clinically effective neuroprotectant has not yet been developed. Numerous neuroprotection trials in acute stroke have failed[1].

Many of these early efforts, however, were focused on targets that might have short half-lives after stroke onset, such as excitotoxicity. Because it is often difficult to reach an individual quickly after stroke onset, such therapies may be difficult to implement widely.

Emerging data implicate a role for inflammation in stroke[2]. In contrast to acute excitotoxic and oxidative stress, prolonged inflammation may offer a longer window of opportunity to block secondary events that expand infarction and brain injury. Inflammation after cerebral ischemia amplifies the initial injury by linking acute responses in glia and cytokines to a sec-

Eng H. Lo is in the Neuroprotection Research Laboratory, Massachusetts General Hospital, Charlestown, Massachusetts, USA.
e-mail: lo@helix.mgh.harvard.edu