Fundamental Pathways in Breast Cancer 4: Signaling to Chromatin in Breast Development

4

Luca Mazzarella and Pier Giuseppe Pelicci

4.1 Breast Development and Its Relationship with Cancer

4.1.1 Normal Breast Development and the Identification of Mammary Stem Cells

Development of the breast has important differences compared to other organs, as it is mostly completed in the postnatal life (for extensive review of breast development, see [1]). At birth, the breast consists only of a rudimentary ductal structure populating the area around the nipple. During puberty and in response to ovarian hormones, a branched ductal architecture develops, driven by highly proliferative "terminal end bud" (TEB) cells. This process is macroscopically and molecularly similar to the epithelial-to-mesenchymal transition (EMT) that occurs earlier in embryonal development and implies infiltration of epithelial cells into the underlying fibroadipose stroma. Postpubertal mammary ducts so formed consist of a bilayer of polarized **luminal** cells surrounded by myoepithelial **basal** cells; small milk-producing alveoli with apocrine **lobular** cells develop; lastly, a specialized stroma with trophic function surrounds the mature gland. Ductal and alveolar cells undergo periodic waves of apoptosis and regeneration at each estrous cycle. During pregnancy and then lactation, duct arborization and alveolar volume increase dramatically through active cell proliferation.

The cell types and molecular mechanisms governing breast regeneration are still incompletely understood. Early experiments showed that any portion of the mammary gland transplanted into a suitable environment (cleared mammary fat pad for mouse-to-mouse transplants, kidney capsule for human-to-mouse) is able to regenerate a functional mammary gland [2, 3]. However, it was unclear whether this ability is shared by most cells or is restricted to few mammary stem cells (MaSCs). The latter view is now supported by a large body of experimental evidence (reviewed in [1, 4, 5]), but the heterogeneity of the experimental systems employed has fueled debate about the features of MaSCs. Earlier transplantation-based experiments revealed that single individual cells able to completely regenerate a functional gland and give rise to all mammary lineages can be prospectively identified in the mouse as $CD24^{+}$ or $CD29^{hi}/CD49f^{hi}$ [6–8]. In humans, $CD49^{+}/EpCAM^{low}$ cells or aldehyde dehydrogenase (ALDH)-active cells are enriched for regenerative activity when transplanted in immunocompromised mice [9, 10]. Furthermore, in vitro mammosphere formation assays revealed that regenerative activity is highly enriched in a rare population that cycles infrequently [11, 12]. This led to a dominant model in which mammary regeneration is carried out by few and slow-cycling stem cells with the ability to give rise to all mature breast lineages, in clear resemblance to models of hematopoiesis. However, transplantation is a highly nonphysiological setting in which the revealed developmental potential may not reflect the actual contribution to normal mammary homeostasis. More recent in vivo lineage tracing experiments have established the existence of cells with "true" multipotency but also revealed that multiple reporter alleles can identify multipotent cells with different expression profiles, frequency, and cell cycle dynamics, suggesting a higher than expected heterogeneity [13–15]. A significant part of adult mammary lineages is replenished by self-renewing "progenitor" cells whose contribution is restricted to the luminal or basal lineages [16, 17]. A subset of self-renewing cells is retained through rounds of pregnancy-induced alveolar remodeling and re-initiates alveologenesis at subsequent pregnancies in mice ("parity-induced mammary epithelial cells," PI-MEC) [18–21], although the existence of such cells has never been demonstrated in humans [4]. It is still unclear whether multipotency is a fixed feature of cells which are multipotent by transplantation *and* by lineage tracing, or whether unipotent

L. Mazzarella (✉) • P.G. Pelicci
Milan, Italy
e-mail: luca.mazzarella@ieo.eu; piergiuseppe.pelicci@ieo.eu

U. Veronesi et al. (eds.), *Breast Cancer*, DOI 10.1007/978-3-319-48848-6_4

progenitors might become multipotent upon strong changes in environmental signals, like physiological hormonal changes or transplantation.

4.1.2 Relationship Between Normal Breast Development and Breast Cancer

The study of normal developmental dynamics can provide important information to understand breast cancer natural history. What is the cell of origin of breast cancer? Multiple cumulative genetic abnormalities are required to transform a normal cell. In the breast, on average one mutation per coding megabase are identified in large-scale sequencing projects [22, 23]. As the rate of mutation accumulation at each cell division is low [24], cells of cancer origin are likely to have a long proliferation history: MaSCs or progenitors surviving multiple cycles of gland regeneration are the likeliest candidates.

But does this also imply that the developmental potential of the cell of origin can shape cancer phenotype? This was strongly suggested by the landmark expression microarray studies of the early 2000s [25, 26], which showed that groups of commonly expressed genes that define normal lineages are also able to define clusters of cancers with common natural history ("intrinsic" subtypes). However transcriptional similarities do not necessarily extend to the cell of origin, implying for instance that a basal-like cancer had to originate from a basal-restricted cell. In fact, more recent studies on BRCA-deficient mice and humans suggest that basal-like cancers derive from luminal-committed progenitors [9, 27]. The emerging consensus is that luminal progenitors are the likely cell of origin for both luminal and basal-like tumors, whereas MaSCs and/or basal progenitors are the initiators of the rarer claudin-low subtype. The origin of HER2+ tumors is less well understood; data in mice suggest that parity-induced stem cells might be the culprit as their ablation inhibits tumorigenesis in MMTV-neu mice [28], but to what extent this model really mimics human HER2+ tumors is disputable since the murine expression profile is more akin to human luminal tumors [4, 28, 29]. Almost completely undetermined is the cell of origin of lobular and rarer breast cancer subtypes. Transcriptome analysis of "special" subtypes (like mucinous and micropapillary) all clustered in a separate group, revealing transcriptional homogeneity despite morphologic differences, but little about a possible common cell of origin [30]. Lobular cancers showed heterogeneous signatures resembling ductal subtypes (including luminal-like and basal-like signatures) [30]; transcriptome signatures within lobular cancers have been recently studied in more depth by the TCGA, with the definition of three new subgroups "reactive-like," "immune-related," and "proliferative" [31]. Again this reveals little about the cell of origin, but the only available genetically engineered mouse model showed that lobular tumors can be obtained from basal cells expressing cytokeratin 14 [32].

4.2 Signaling Pathways Controlling Breast Development and Their Alterations in Cancer

Several authors have proposed that molecular mechanisms specific for stem cells might also be responsible for malignant behavior, in a declination of the "cancer stem cell" theory [33]. Specifically, the same pathways allowing stem cells to maintain multilineage potential resist to physiological death and invade the stroma to (re)generate the organ, might also allow cancer cells to adapt their transcriptome, resist to treatment-induced death, and become invasive and metastatic. We will now review the most important developmental and stem cell-related pathways in breast. Then, we will review how these signals converge on chromatin (the ensemble of normal DNA and DNA-bound proteins) to modulate transcription. Chromatin can directly (by physical compaction) or indirectly (through differential recruitment of effector proteins) control DNA accessibility to transcription factors and stabilize phenotypic traits by restricting the degree to which transcriptional signatures can be further modified by competing signals. Hence, proteins directly governing chromatin architecture are particularly important in developmental processes and are often disrupted in cancer.

4.2.1 NOTCH/NUMB and p53 as Regulators of Symmetric vs Asymmetric Cell Division

4.2.1.1 NOTCH Pathway in Breast Development

The NOTCH pathway governs cell lineage determination and body patterning in all metazoans [34–36]. NOTCH was classically identified as a regulator of neuroectodermal development in *Drosophila* but then emerged as a functional module repeatedly exploited in heterogeneous developmental contexts to execute binary cell fate choices, generating and maintaining phenotypic "boundaries" within organs. The four NOTCH receptors are functionally redundant [37] transmembrane proteins with homology to eGFR; once activated by short-range ligand binding, usually requiring cell-to-cell interaction, the receptor is cleaved by the gamma-secretase complex and transported into the nucleus. Here, it induces the assembly of a highly conserved protein complex that canonically includes CBF-1 and RBP-J, plus additional chromatin-modifying enzymes [38]; this drives transcription of NOTCH target genes, most notably the HES and HERP families of bHLH transcription factors [39, 40].

The half-life of the activated receptor is normally very short due to efficient proteasomal degradation, which is dependent on the C-terminal PEST (rich in proline (P), glutamic acid (E), serine (S), and threonine (T)) domain common to all NOTCH receptors. The NOTCH molecular circuitry is reinforced by multiple layers of intrinsic and extrinsic feedback mechanisms ("lateral inhibition") that amplify small variations in ligand/receptor concentrations between adjacent cells; this allows the emergence of discontinuities among cell populations with prior equal potentials. A particularly important regulatory role is played by NUMB, a membrane-associated protein that targets NOTCH receptors to proteasomal degradation and is a key determinant of asymmetric cell division: in several NOTCH-dependent lineage choices, NUMB is unequally partitioned between daughter cells, leading to differential inactivation of the NOTCH pathway. Numb also independently regulates the stability of p53 [41], which in itself is implicated in stem cell self-renewal and (a) symmetric cell division [11].

These general functional properties also apply to NOTCH role in breast biology. NOTCH pathway receptors are expressed in the luminal compartment [42–44], and its target genes are upregulated in luminal progenitors in the adult gland [42]. This expression pattern is mutually exclusive with that of ΔNp63, which promotes and maintains basal cell gene expression; in fact, NOTCH and p63 appear to be functional antagonists [45]. Hyperactivation of NOTCH pathway by overexpression of the active form [42] or conditional ablation of NUMB [44] leads to ductal hyperplasia with luminal differentiation. On the contrary, ablation of NOTCH function by conditional deletion of the common transcriptional mediators CBF1 or RBP-J led to expansion of the basal cell pool during pregnancy [46]. Collectively, these results suggest a model in which NOTCH pathway activation promotes the commitment of dividing stem cells and progenitors to the luminal lineage at the expenses of the basal/myoepithelial lineage [47]. However, this model is complicated by the presence of low levels of NUMB and NOTCH receptors also in basal and other cell types [42, 44]; the precise mechanism of action of NOTCH in normal mammary gland biology remains an active field of research.

4.2.1.2 Modes of NOTCH Pathway Alterations in Breast Cancer

Although altered NOTCH receptors have been found to act as tumor suppressors in some circumstances [48, 49], in breast cancer and in most other tumors (most notably T-ALL), they behave as classical proto-oncogenes that become constitutively activated through loss of extrinsic or intrinsic regulation [49, 50].

Loss of extrinsic regulation is achieved by genetic ablation of the N-terminal extracellular domain. Early on in the history of breast cancer experimental research, this mechanism was identified as a consequence of insertional mutagenesis of the mouse mammary tumor virus; breast-specific expression of the truncated form induces expansion of luminal progenitors and mammary tumors in experimental animals [51–53].

Among human tumors, alterations in NOTCH receptors are present in around 5% of all cases in different patient populations. Unlike MMTV insertions in the mouse, the generation of an extracellular domain-defective protein is uncommon in humans; this was observed as the result of chromosomal translocations in a recent study [54, 55]. More common are point mutations that frequently (around 60% in the TCGA cohort) are truncating and clustered at 3′ exons, resulting in a disrupted PEST domain and predicted to lead to increased protein half-life. The PEST can also be lost through deletions or translocations [54, 55]. The remaining point mutations are scattered throughout the gene body with no detectable pattern but tend to occur in highly conserved residues important for receptor heterodimerization associated with increased activity in T-ALL [54]. NUMB is frequently downregulated at the protein level in breast cancer, although the mechanism leading to downregulation has not been extensively studied. Deletions can be observed in 0.6% of all TCGA breast cancer patients.

NOTCH/NUMB alterations are strongly associated with HER2/ER/PgR negativity [41, 54, 56] and, as expected, with unfavorable outcome in invasive carcinoma [41, 43, 56–59] and with higher recurrence rate in DCIS [60]. This makes NOTCH pathway an attractive target for drug development. Inhibiting NOTCH through genetic [61, 62] or pharmacological [60, 63] means results in a loss of in vitro self-renewal ability in mammosphere assays.

NOTCH inhibitors are currently undergoing early phase clinical evaluation in breast cancer and other tumors. Two main approaches are being explored: the use of antibodies that disrupt ligand-receptor interaction and inhibitors of the gamma-secretase activity first explored in Alzheimer's disease [64–66].

4.2.2 The Wingless (WNT) Pathway

4.2.2.1 Wnt Pathway in Breast Development

The Wingless (WNT) pathway plays a crucial role in mammary development; similarly to NOTCH, it acts prevalently at short range as a functional module that is repeatedly used in highly different contexts to give rise to variable outputs, including the regulation of asymmetric cell division. Signals are instructed through paracrine cellular communication between the lipidated Wnt ligands and the Frizzled transmembrane receptors. This results in the phosphorylation of the canonical WNT mediator beta-catenin by casein kinase I (CKI) and glycogen synthase-3β (GSK-3β), resulting in its stabilization and nuclear translocation. In the nucleus,

beta-catenin activates transcription of conserved targets, namely, telomerase, Axin2, and LGR5, through TCF/LEF factors. Signal strength is intrinsically regulated through proteasomal degradation of Frizzled receptors by Rnf43/Znrf3, which is in turn inhibited by LGR5; LGR5 marks WNT-responsive cells in a variety of epithelial tissues [67], and in the breast it marks a subset of bipotential stem cells able to give rise to luminal and myoepithelial cells as defined by lineage tracing experiments [13]. PROCR is another WNT target that also marks multipotent mammary stem cells, although intriguingly, PROCR$^+$ cells appear distinct from LGR5$^+$ [14].

Another level of WNT modulation is through sequestration of beta-catenin to adherent junctions by E-cadherin: this peripheral pool of beta-catenin is unavailable for nuclear translocation and is thought to play a role in epithelial-to-mesenchymal transitions [68, 69].

4.2.2.2 Modes of WNT Pathway Alterations in Breast Cancer

Similarly to NOTCH, the Wnt1 receptor was found early on as a common MMTV integration site [70], and the oncogenic potential of Wnt hyperactivation subsequently demonstrated by MMTV-induced overexpression of several Wnt receptors or of beta-catenin [71–75]. This leads to anticipated lobuloalveolar overgrowth, morphologically similar to that induced by pregnancy but with an expansion of poorly differentiated cells [73]; importantly, this is also true in male mice and upon transplantation into ovariectomized recipients, suggesting that Wnt pathway lies downstream of ovarian hormones and that Wnt-aberrant cells might become estrogen independent [76, 77]. As mice age, invasive ductal tumors develop with a penetrance of 100% by 1 year. The long penetrance suggests that additional mutations are required to achieve the invasive phenotype, but importantly ablation of Wnt signaling is still required after the invasive tumor has formed, although loss of p53 facilitates the transition to WNT independence [78].

WNT-hyperactivated mouse models have been used extensively in basic research, but their relevance to clinical practice might be questionable, since components of the canonical Wnt pathway are not frequently mutated in breast cancer [76, 79]. However, aberrant beta-catenin staining patterns (i.e., prevalence of nuclear pattern) is observed in about 20% of ductal carcinomas and, as it might be expected, is correlated with triple-negative histology and poor prognosis [79, 80]. Aberrant beta-catenin expression is also correlated with lobular histology, given its association with E-cadherin loss [79, 80].

The absence of a clear targetable alteration made WNT an attractive but difficult pathway for drug development. Recently, casein kinase 1d (CK1d) was found to be amplified and overexpressed in strong correlation with WNT pathway genes in 36% breast cancers, particularly in luminal B and triple-negative ones. The CK1D inhibitor SR-3029 was highly effective in preclinical models (orthotopic cell line transplantation) [81].

4.2.3 Inducers of Epithelial-to-Mesenchymal Transition

Invasion of epithelial cells into connective and adipose tissue is a physiological phase of pubertal breast development and is governed by signaling pathways that have also been implicated in the acquisition of metastatic potential. This process bears resemblance to the physiological epithelial-to-mesenchymal transition (EMT) that occurs during crucial phases of embryogenesis like gastrulation. Whether tumoral invasion is truly an aberrant form of EMT has been a matter of dispute, mostly due to the fact that normal EMT implies dramatic morphological and molecular transitions that have not been consistently observed in breast and other tumors. However, a recent study showed that highly sensitive analysis of pathological specimens can identify cells with mixed epithelial/mesenchymal markers in invasive but not noninvasive breast cancers. These cells correlate with primary histology (mostly triple negative) and can be found circulating in proportions that vary according to treatment response [82].

The exact wiring of the signaling circuits responsible for EMT-like responses in breast cancer has not been fully worked out. Overexpression of specific individual transcription factors (SNAIL, TWIST, SLUG, and ZEB1/2) is able to initiate EMT and increase invasiveness in noninvasive breast cells [83–87]. Several extracellular signals are also implicated, most notably transforming growth factor beta (TGFb), WNT, and Sonic Hedgehog, the latter in turn activated by FOXC1/2 and the basal cell-specific p63 [88]. A common outcome of EMT response is the loss of E-cadherin expression, which results in a weakening of cell-to-cell adhesion and the release of a cytoplasmic pool of beta-catenin, which can now enter in WNT-dependent regulation. A second, recently discovered output of EMT activation is the activation of the Hippo pathway, which in breast cancer is correlated with metastatic behavior and resistance to chemotherapy [89, 90].

All these pathways are rarely affected by genetic aberrations in breast cancer but appear frequently deregulated through nongenetic mechanisms in poor-prognosis breast tumors, especially of the basal and claudin-low subtype [91, 92]. As such, they have attracted attention as drug targets [93] but are still limited to preclinical development.

4.2.4 GATA3, FOXA1, and Lobular vs Ductal Tumors

GATA3 and FOXA1 are both implicated in the regulation of estrogen-mediated transcription (see chapter by Magnani), and their expression is strongly associated with estrogen positivity in tumors [94]. If conditionally deleted during puberty or adult life, they abrogate or severely distort

mammary gland development, with loss of luminal cell identity in the case of GATA3 [95, 96] and a block in terminal end bud formation and invasion during puberty for FOXA1 [97]. Their functional similarities extend to their molecular mode of action, as both are so-called "pioneering" factors able to condition chromatin structure and subsequent binding of other transcription factors [98]. FOXA1, ER, and GATA3 physically interact with several other chromatin regulators in a "mega transcription factor complex" nucleated by the estrogen receptor in response to estradiol stimulation. As FOXA1 directly promotes ER expression, these three factors form a regulatory network able to stabilize estrogen-dependent transcription [99].

Intriguingly, the mutational pattern of GATA3 and FOXA1 has recently emerged as mutually exclusive in the two forms of strongly ER+ breast cancers: GATA3 is frequently mutated in ductal luminal cancers, while FOXA1 is as frequently mutated in lobular cancers [31].

Mutations in GATA3 are the third most common alteration in breast cancer globally [100]. SNVs are invariably heterozygous and cluster in three specific categories: splice site mutations at the junctions between exons 4/5 and 5/6 (20%), frameshift mutations in exon 6 (50%), and frameshifts in zinc finger 2 (10%). Also, GATA3 is frequently amplified (28% of all GATA3 alterations in the TCGA dataset), but this has received little attention. Mutation type 3 is the only type that has been characterized molecularly [101, 102]. Although SNVs cause apparent GATA3 loss of function, they appear to stabilize the non-mutated allele, leading to an intriguing model that can explain the requirement for maintaining heterozygosity [103]. The frequently mutated MAP3K1 is also a target of GATA3, and recently a germline variant in its GATA3-bound promoter was discovered in a genome-wide association study [104]. Loss of GATA3 expression correlates with acquisition of metastatic potential in the MMTV-PyMT mouse model of luminal cancer [105].

Mutations in FOXA1 cluster on lysines located on the wings of the Forkhead domain. These residues, when acetylated by EP300, prevent DNA binding; thus, their loss creates a strongly bound FOXA1 at sites of ER binding, amplifying a normally estrogen-dependent response on the absence of the hormone [31].

4.3 Chromatin Marks in Normal and Neoplastic Breast

The study of chromatin factors in breast development and cancer is probably less advanced than in other systems. In hematopoiesis and its malignancies, where mutations in chromatin factors were identified first, targeted drugs have already made it to the clinic and are routinely used [106]. We will skip lengthy discussions on basic chromatin structure, for which the reader is addressed to extensive reviews [107, 108] Proteins involved in interactions with chromatin have been functionally divided in writers, erasers, and readers [109], a useful classification that will be maintained here. We will focus on those aspects of chromatin regulation not specifically related to estrogen receptor biology, which is extensively covered by L. Magnani in this book (ref).

4.3.1 DNA Methylation

DNA methylation dynamics and the role of DNA methyltransferases in normal breast development have not been extensively investigated. In most breast tumors, primitive techniques could identify gross aberrations in DNA methylation as compared to normal tissues. Locus-specific analyses carried out at relevant genes (e.g., estrogen receptor) could also show aberrant methylation, but the relevance of this information has remained questionable; only recently genome-wide investigations have been systematically applied to large patient cohorts in the TCGA [100] and other studies [110, 111]. Unfortunately, even these systematic studies are complicated by the still poorly understood relationship with gene expression, and by significant heterogeneity in the techniques employed, none of which is truly able to fully cover all potentially methylated cytosines. In the TCGA, basal-like tumors tended to be globally hypermethylated, and, importantly, BRCA1 hypermethylation appeared to be a frequent mechanism (24%) for gene downregulation, potentially suggesting an involvement of BRCA functional loss in the absence of genetic alterations. A group of MSKCC performed a bioinformatically more refined analysis on 171 samples of heterogeneous histology and identified a cluster of tumors with methylation profile similar to that identified in colon cancer ("breast CpG island methylator profile," B-CIMP), which was associated with significantly lower propensity to metastasize. Different results were obtained by an Australian group focusing on triple-negative cancers; here, hypomethylated tumors were associated with better prognosis.

4.3.2 Histone Modifications

Histone modifications in normal breast cell populations have been systematically studied by Polyak et al. [112], who focused on the two marks that define actively transcribed and repressed genomic regions: trimethylation of H3K4 and H3K27. Regions where both signals overlap ("bivalent chromatin") are considered to be epigenetically plastic and

enriched in multipotent stem cells at genes with strong lineage-defining activity [113, 114]. $CD24^+$ and $CD44^+$ cells showed a different distribution of these marks, and many lineage-defining genes, especially transcription factors, were shown to maintain chromatin bivalency, suggesting a basis for phenotypic plasticity. In particular, ZEB transcription factors are bivalent in some tumor cell lines and regulate the expression of CD44, a marker of cancer stem cells with increased invasiveness. Robert Weinberg and colleagues demonstrated that cells in which ZEB1 is bivalent (but CD44 is repressed, like in luminal cancer cell lines) are able to resolve bivalency and lose the repressive H3K27me3 in response to TGFb, resulting in CD44 upregulation and acquisition of invasive traits [115], directly linking an extracellular stimulus with a chromatin-mediated phenotypic change. The Polyak lab investigated further the role of chromatin in phenotypic reprogramming. Using elegant cell fusion experiments between cell lines with luminal or basal features, they showed that the basal phenotype is dominant over the luminal and can be induced by even short-term exposure of luminal cells to basal cell total extracts; reprogramming correlated with the acquisition of epigenetic traits of the parental basal cell, in particular the super-enhancer profile defined by elevated H3K27ac [116]. It might be interesting to explore whether luminal-to-basal epigenetic reprogramming is at the basis of estrogen expression discordance between primary and relapsed tumors, which more often become estrogen receptor negative from positive than vice versa [117].

4.3.3 Chromatin Writers

Members of the Polycomb family (so called from the developmental phenotype observed in *Drosophila* mutants) are the best-studied chromatin writers in breast development. Polycomb proteins are organized in two sets of complexes which induce histone modifications associated with gene repression, namely, H2AK119 ubiquitylation (Polycomb repressive complex 1, PRC1) and H3K27 methylation (PRC2). Members of both complexes have been found to play a role in breast development and cancer [118–122]. Polycomb factors are involved in the maintenance of pluripotency in most if not all stem cells in adult and embryonal life; their genetic disruption leads to increased transcriptional plasticity at lineage-specific genes, resulting in a failure to coordinately execute differentiation programs. The ultimate outcome of Polycomb ablation is highly variable depending on the examined system and can result in cell death. Polycomb inhibitors, especially those directed against the PRC2 catalytic subunit EZH2 that is overexpressed and correlated with poor prognosis in breast cancer [123, 124], have shown responses in preclinical studies [125–127].

4.3.4 Chromatin Erasers

Factors that remove histone acetylation and methylation maintain chromatin in a dynamic state, making it more or less amenable to transcriptional changes. Many cancer cells depend on persistent deacetylase or demethylase activity for survival, and their ablation can lead to cell death or differentiation. Given their favorable chemical properties for drug design, chromatin erasers have been identified as interesting targets for drug development. Histone deacetylases (HDACs) were identified first as playing a role in breast cancer [128], especially by virtue of their negative effect on estrogen receptor expression [129]; thus, they have been mostly studied as sensitizers to endocrine therapy [130–132]. Of the several inhibitors with different degree of specificity synthesized so far, entinostat has reached the furthest clinical development, showing efficacy in a randomized phase II trial against placebo in combination with exemestane in aromatase inhibitor-refractory advanced ER+ breast cancer [133].

Recent research also revealed important roles for histone demethylases. The H3K4 demethylase JARID1B (also known as KDM5B), involved in mammary gland development and GATA3 recruitment to FOXA1 promoter [134], was found frequently amplified and overexpressed in multiple breast cancers, particularly in luminal cancers where overexpression of JARID1B target genes identified a subset of patients with poorer survival [135]. Another demethylase, LSD1 (also known as KDM1A), which targets H3K4 mono- and di-methylation and is involved in enhancer "decommissioning" during cell differentiation [136], was found to regulate breast cancer metastasis [137]. LSD1 inhibitors are in early clinical trials but have not been yet tested in breast cancers.

4.3.5 Chromatin Readers

Chromatin "readers" are proteins with domains able to recognize specific chromatin modifications and guide locus-specific assembly of transcription regulator complexes. A relevant example in breast biology is the Pygo2 factor that contains the PHD finger domain able to recognize H3K4me3. Pygo2 is a crucial transducer of WNT signals in breast development [138] and is essential for the survival of several breast cancer cell lines [139]. Disruption of chromatin interaction is a novel pharmacological strategy that has yielded intriguing results when targeted against bromodomain-containing proteins. These interact with acetylated histones and are important factors for super-enhancer activity. As super-enhancers are associated with highly tissue- or cancer-specific transcription [140], their targeting might benefit from an elevated therapeutic index. BET inhibitors, of which JQ1 is the progenitor, are undergoing rapid drug development and have recently shown particularly promising activity in triple-negative breast cancer [141].

Conclusions

Full understanding of the molecular pathways described in this chapter will require an elevated degree of integration between developmental biology, biochemistry, and epigenomics. The benefits that can be reaped for patients are high, as targeting differentiation and stem cells may lead to durable responses or even disease eradication, unachievable with drugs targeting proliferation or genome stability. Technological advancements of the last decade have made this endeavor realistic.

References

1. Inman JL, Robertson C, Mott JD, Bissell MJ (2015) Mammary gland development: cell fate specification, stem cells and the microenvironment. Development 142(6):1028–1042. doi:10.1242/dev.087643
2. Deome KB, Faulkin LJ Jr, Bern HA, Blair PB (1959) Development of mammary tumors from hyperplastic alveolar nodules transplanted into gland-free mammary fat pads of female C3H mice. Cancer Res 19(5):515–520
3. Daniel CW, Deome KB, Young JT, Blair PB, Faulkin LJ Jr (2009) The in vivo life span of normal and preneoplastic mouse mammary glands: a serial transplantation study. 1968. J Mammary Gland Biol Neoplasia 14(3):355–362. doi:10.1007/s10911-009-9139-3
4. Skibinski A, Kuperwasser C (2015) The origin of breast tumor heterogeneity. Oncogene 34(42):5309–5316. doi:10.1038/onc.2014.475
5. Sreekumar A, Roarty K, Rosen JM (2015) The mammary stem cell hierarchy: a looking glass into heterogeneous breast cancer landscapes. Endocr Relat Cancer 22(6):T161–T176. doi:10.1530/ERC-15-0263
6. Kordon EC, Smith GH (1998) An entire functional mammary gland may comprise the progeny from a single cell. Development 125(10):1921–1930
7. Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML et al (2006) Generation of a functional mammary gland from a single stem cell. Nature 439(7072):84–88. doi:10.1038/nature04372
8. Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, Choi D et al (2006) Purification and unique properties of mammary epithelial stem cells. Nature 439(7079):993–997. doi:10.1038/nature04496
9. Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH et al (2009) Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. Nat Med 15(8):907–913. doi:10.1038/nm.2000
10. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M et al (2007) ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 1(5):555–567. doi:10.1016/j.stem.2007.08.014
11. Cicalese A, Bonizzi G, Pasi CE, Faretta M, Ronzoni S, Giulini B et al (2009) The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. Cell 138(6):1083–1095. doi:10.1016/j.cell.2009.06.048
12. Dontu G, Abdallah WM, Foley JM, Jackson KW, Clarke MF, Kawamura MJ et al (2003) In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. Genes Dev 17(10):1253–1270. doi:10.1101/gad.1061803
13. Rios AC, Fu NY, Lindeman GJ, Visvader JE (2014) In situ identification of bipotent stem cells in the mammary gland. Nature 506(7488):322–327. doi:10.1038/nature12948
14. Wang D, Cai C, Dong X, Yu QC, Zhang XO, Yang L et al (2015) Identification of multipotent mammary stem cells by protein C receptor expression. Nature 517(7532):81–84. doi:10.1038/nature13851
15. dos Santos CO, Rebbeck C, Rozhkova E, Valentine A, Samuels A, Kadiri LR et al (2013) Molecular hierarchy of mammary differentiation yields refined markers of mammary stem cells. Proc Natl Acad Sci U S A 110(18):7123–7130. doi:10.1073/pnas.1303919110
16. Prater MD, Petit V, Alasdair Russell I, Giraddi RR, Shehata M, Menon S et al (2014) Mammary stem cells have myoepithelial cell properties. Nat Cell Biol 16(10):942–950, 1–7. doi:10.1038/ncb3025
17. Van Keymeulen A, Rocha AS, Ousset M, Beck B, Bouvencourt G, Rock J et al (2011) Distinct stem cells contribute to mammary gland development and maintenance. Nature 479(7372):189–193. doi:10.1038/nature10573
18. Chang TH, Kunasegaran K, Tarulli GA, De Silva D, Voorhoeve PM, Pietersen AM (2014) New insights into lineage restriction of mammary gland epithelium using parity-identified mammary epithelial cells. Breast Can Res 16(1):R1. doi:10.1186/bcr3593
19. Boulanger CA, Wagner KU, Smith GH (2005) Parity-induced mouse mammary epithelial cells are pluripotent, self-renewing and sensitive to TGF-beta1 expression. Oncogene 24(4):552–560. doi:10.1038/sj.onc.1208185
20. Lo PK, Kanojia D, Liu X, Singh UP, Berger FG, Wang Q et al (2012) CD49f and CD61 identify Her2/neu-induced mammary tumor-initiating cells that are potentially derived from luminal progenitors and maintained by the integrin-TGFbeta signaling. Oncogene 31(21):2614–2626. doi:10.1038/onc.2011.439
21. Wagner KU, Boulanger CA, Henry MD, Sgagias M, Hennighausen L, Smith GH (2002) An adjunct mammary epithelial cell population in parous females: its role in functional adaptation and tissue renewal. Development 129(6):1377–1386
22. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV et al (2013) Signatures of mutational processes in human cancer. Nature 500(7463):415–421. doi:10.1038/nature12477
23. Lawrence MS, Stojanov P, Polak P, Kryukov GV, Cibulskis K, Sivachenko A et al (2013) Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 499(7457):214–218. doi:10.1038/nature12213
24. Makova KD, Hardison RC (2015) The effects of chromatin organization on variation in mutation rates in the genome. Nat Rev Genet 16(4):213–223. doi:10.1038/nrg3890
25. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA et al (2000) Molecular portraits of human breast tumours. Nature 406(6797):747–752. doi:10.1038/35021093
26. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A et al (2003) Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 100(14):8418–8423. doi:10.1073/pnas.0932692100
27. Molyneux G, Geyer FC, Magnay FA, McCarthy A, Kendrick H, Natrajan R et al (2010) BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells. Cell Stem Cell 7(3):403–417. doi:10.1016/j.stem.2010.07.010
28. Henry MD, Triplett AA, Oh KB, Smith GH, Wagner KU (2004) Parity-induced mammary epithelial cells facilitate tumorigenesis in MMTV-neu transgenic mice. Oncogene 23(41):6980–6985. doi:10.1038/sj.onc.1207827
29. Pfefferle AD, Herschkowitz JI, Usary J, Harrell JC, Spike BT, Adams JR et al (2013) Transcriptomic classification of genetically engineered mouse models of breast cancer identifies human subtype counterparts. Genome Biol 14(11):R125. doi:10.1186/gb-2013-14-11-r125
30. Weigelt B, Horlings HM, Kreike B, Hayes MM, Hauptmann M, Wessels LF et al (2008) Refinement of breast cancer classification by molecular characterization of histological special types. J Pathol 216(2):141–150. doi:10.1002/path.2407

31. Ciriello G, Gatza ML, Beck AH, Wilkerson MD, Rhie SK, Pastore A et al (2015) Comprehensive molecular portraits of invasive lobular breast cancer. Cell 163(2):506–519. doi:10.1016/j.cell.2015.09.033
32. Derksen PW, Liu X, Saridin F, van der Gulden H, Zevenhoven J, Evers B et al (2006) Somatic inactivation of E-cadherin and p53 in mice leads to metastatic lobular mammary carcinoma through induction of anoikis resistance and angiogenesis. Cancer Cell 10(5):437–449. doi:10.1016/j.ccr.2006.09.013
33. Visvader JE, Lindeman GJ (2012) Cancer stem cells: current status and evolving complexities. Cell Stem Cell 10(6):717–728. doi:10.1016/j.stem.2012.05.007
34. Ranganathan P, Weaver KL, Capobianco AJ (2011) Notch signalling in solid tumours: a little bit of everything but not all the time. Nat Rev Cancer 11(5):338–351. doi:10.1038/nrc3035
35. Fortini ME (2009) Notch signaling: the core pathway and its post-translational regulation. Dev Cell 16(5):633–647. doi:10.1016/j.devcel.2009.03.010
36. Gazave E, Lapebie P, Richards GS, Brunet F, Ereskovsky AV, Degnan BM et al (2009) Origin and evolution of the notch signalling pathway: an overview from eukaryotic genomes. BMC Evol Biol 9:249. doi:10.1186/1471-2148-9-249
37. Kitamoto T, Takahashi K, Takimoto H, Tomizuka K, Hayasaka M, Tabira T et al (2005) Functional redundancy of the notch gene family during mouse embryogenesis: analysis of notch gene expression in Notch3-deficient mice. Biochem Biophys Res Commun 331(4):1154–1162. doi:10.1016/j.bbrc.2005.03.241
38. Arnett KL, Hass M, McArthur DG, Ilagan MX, Aster JC, Kopan R et al (2010) Structural and mechanistic insights into cooperative assembly of dimeric notch transcription complexes. Nat Struct Mol Biol 17(11):1312–1317. doi:10.1038/nsmb.1938
39. Borggrefe T, Oswald F (2009) The notch signaling pathway: transcriptional regulation at notch target genes. Cell Mol Life Sci 66(10):1631–1646. doi:10.1007/s00018-009-8668-7
40. Iso T, Kedes L, Hamamori Y (2003) HES and HERP families: multiple effectors of the notch signaling pathway. J Cell Physiol 194(3):237–255. doi:10.1002/jcp.10208
41. Colaluca IN, Tosoni D, Nuciforo P, Senic-Matuglia F, Galimberti V, Viale G et al (2008) NUMB controls p53 tumour suppressor activity. Nature 451(7174):76–80. doi:10.1038/nature06412
42. Bouras T, Pal B, Vaillant F, Harburg G, Asselin-Labat ML, Oakes SR et al (2008) Notch signaling regulates mammary stem cell function and luminal cell-fate commitment. Cell Stem Cell 3(4):429–441. doi:10.1016/j.stem.2008.08.001
43. Pece S, Serresi M, Santolini E, Capra M, Hulleman E, Galimberti V et al (2004) Loss of negative regulation by numb over notch is relevant to human breast carcinogenesis. J Cell Biol 167(2):215–221. doi:10.1083/jcb.200406140
44. Tosoni D, Zecchini S, Coazzoli M, Colaluca I, Mazzarol G, Rubio A et al (2015) The numb/p53 circuitry couples replicative self-renewal and tumor suppression in mammary epithelial cells. J Cell Biol 211(4):845–862. doi:10.1083/jcb.201505037
45. Yalcin-Ozuysal O, Fiche M, Guitierrez M, Wagner KU, Raffoul W, Brisken C (2010) Antagonistic roles of notch and p63 in controlling mammary epithelial cell fates. Cell Death Differ 17(10):1600–1612. doi:10.1038/cdd.2010.37
46. Buono KD, Robinson GW, Martin C, Shi S, Stanley P, Tanigaki K et al (2006) The canonical notch/RBP-J signaling pathway controls the balance of cell lineages in mammary epithelium during pregnancy. Dev Biol 293(2):565–580. doi:10.1016/j.ydbio.2006.02.043
47. Raouf A, Zhao Y, To K, Stingl J, Delaney A, Barbara M et al (2008) Transcriptome analysis of the normal human mammary cell commitment and differentiation process. Cell Stem Cell 3(1):109–118. doi:10.1016/j.stem.2008.05.018
48. Viatour P, Ehmer U, Saddic LA, Dorrell C, Andersen JB, Lin C et al (2011) Notch signaling inhibits hepatocellular carcinoma following inactivation of the RB pathway. J Exp Med 208(10):1963–1976. doi:10.1084/jem.20110198
49. Lobry C, Oh P, Aifantis I (2011) Oncogenic and tumor suppressor functions of notch in cancer: it's NOTCH what you think. J Exp Med 208(10):1931–1935. doi:10.1084/jem.20111855
50. Weng AP, Ferrando AA, Lee W, Morris JP, Silverman LB, Sanchez-Irizarry C et al (2004) Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 306(5694):269–271. doi:10.1126/science.1102160
51. Robbins J, Blondel BJ, Gallahan D, Callahan R (1992) Mouse mammary tumor gene int-3: a member of the notch gene family transforms mammary epithelial cells. J Virol 66(4):2594–2599
52. Gallahan D, Jhappan C, Robinson G, Hennighausen L, Sharp R, Kordon E et al (1996) Expression of a truncated Int3 gene in developing secretory mammary epithelium specifically retards lobular differentiation resulting in tumorigenesis. Cancer Res 56(8):1775–1785
53. Capobianco AJ, Zagouras P, Blaumueller CM, Artavanis-Tsakonas S, Bishop JM (1997) Neoplastic transformation by truncated alleles of human NOTCH1/TAN1 and NOTCH2. Mol Cell Biol 17(11):6265–6273
54. Wang K, Zhang Q, Li D, Ching K, Zhang C, Zheng X et al (2015) PEST domain mutations in notch receptors comprise an oncogenic driver segment in triple-negative breast cancer sensitive to a gamma-secretase inhibitor. Clin Cancer Res 21(6):1487–1496. doi:10.1158/1078-0432.CCR-14-1348
55. Robinson DR, Kalyana-Sundaram S, Wu YM, Shankar S, Cao X, Ateeq B et al (2011) Functionally recurrent rearrangements of the MAST kinase and notch gene families in breast cancer. Nat Med 17(12):1646–1651. doi:10.1038/nm.2580
56. Rennstam K, McMichael N, Berglund P, Honeth G, Hegardt C, Ryden L et al (2010) Numb protein expression correlates with a basal-like phenotype and cancer stem cell markers in primary breast cancer. Breast Cancer Res Treat 122(2):315–324. doi:10.1007/s10549-009-0568-x
57. Lee CW, Simin K, Liu Q, Plescia J, Guha M, Khan A et al (2008) A functional notch-survivin gene signature in basal breast cancer. Breast Cancer Res 10(6):R97. doi:10.1186/bcr2200
58. Reedijk M, Odorcic S, Chang L, Zhang H, Miller N, McCready DR et al (2005) High-level coexpression of JAG1 and NOTCH1 is observed in human breast cancer and is associated with poor overall survival. Cancer Res 65(18):8530–8537. doi:10.1158/0008-5472.CAN-05-1069
59. Reedijk M, Pinnaduwage D, Dickson BC, Mulligan AM, Zhang H, Bull SB et al (2008) JAG1 expression is associated with a basal phenotype and recurrence in lymph node-negative breast cancer. Breast Cancer Res Treat 111(3):439–448. doi:10.1007/s10549-007-9805-3
60. Farnie G, Clarke RB, Spence K, Pinnock N, Brennan K, Anderson NG et al (2007) Novel cell culture technique for primary ductal carcinoma in situ: role of notch and epidermal growth factor receptor signaling pathways. J Natl Cancer Inst 99(8):616–627. doi:10.1093/jnci/djk133
61. Sansone P, Storci G, Tavolari S, Guarnieri T, Giovannini C, Taffurelli M et al (2007) IL-6 triggers malignant features in mammospheres from human ductal breast carcinoma and normal mammary gland. J Clin Invest 117(12):3988–4002. doi:10.1172/JCI32533
62. Sansone P, Storci G, Giovannini C, Pandolfi S, Pianetti S, Taffurelli M et al (2007) p66Shc/notch-3 interplay controls self-renewal and hypoxia survival in human stem/progenitor cells of the mammary gland expanded in vitro as mammospheres. Stem Cells 25(3):807–815. doi:10.1634/stemcells.2006-0442

63. Grudzien P, Lo S, Albain KS, Robinson P, Rajan P, Strack PR et al (2010) Inhibition of notch signaling reduces the stem-like population of breast cancer cells and prevents mammosphere formation. Anticancer Res 30(10):3853–3867
64. Andersson ER, Lendahl U (2014) Therapeutic modulation of notch signalling--are we there yet? Nat Rev Drug Discov 13(5):357–378. doi:10.1038/nrd4252
65. Brennan K, Clarke RB (2013) Combining notch inhibition with current therapies for breast cancer treatment. Ther Adv Med Oncol 5(1):17–24. doi:10.1177/1758834012457437
66. Messersmith WA, Shapiro GI, Cleary JM, Jimeno A, Dasari A, Huang B et al (2015) A phase I, dose-finding study in patients with advanced solid malignancies of the oral gamma-secretase inhibitor PF-03084014. Clin Cancer Res 21(1):60–67. doi:10.1158/1078-0432.CCR-14-0607
67. Clevers H, Loh KM, Nusse R (2014) Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. Science 346(6205):1248012. doi:10.1126/science.1248012
68. Nelson WJ, Nusse R (2004) Convergence of Wnt, beta-catenin, and cadherin pathways. Science 303(5663):1483–1487. doi:10.1126/science.1094291
69. Tian X, Liu Z, Niu B, Zhang J, Tan TK, Lee SR et al (2011) E-cadherin/beta-catenin complex and the epithelial barrier. J Biomed Biotechnol 2011:567305. doi:10.1155/2011/567305
70. Nusse R, Varmus HE (1982) Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell 31(1):99–109
71. Imbert A, Eelkema R, Jordan S, Feiner H, Cowin P (2001) Delta N89 beta-catenin induces precocious development, differentiation, and neoplasia in mammary gland. J Cell Biol 153(3):555–568
72. Teuliere J, Faraldo MM, Deugnier MA, Shtutman M, Ben-Ze'ev A, Thiery JP et al (2005) Targeted activation of beta-catenin signaling in basal mammary epithelial cells affects mammary development and leads to hyperplasia. Development 132(2):267–277. doi:10.1242/dev.01583
73. Li Y, Welm B, Podsypanina K, Huang S, Chamorro M, Zhang X et al (2003) Evidence that transgenes encoding components of the Wnt signaling pathway preferentially induce mammary cancers from progenitor cells. Proc Natl Acad Sci U S A 100(26):15853–15858. doi:10.1073/pnas.2136825100
74. Lane TF, Leder P (1997) Wnt-10b directs hypermorphic development and transformation in mammary glands of male and female mice. Oncogene 15(18):2133–2144. doi:10.1038/sj.onc.1201593
75. Tsukamoto AS, Grosschedl R, Guzman RC, Parslow T, Varmus HE (1988) Expression of the int-1 gene in transgenic mice is associated with mammary gland hyperplasia and adenocarcinomas in male and female mice. Cell 55(4):619–625
76. Brennan KR, Brown AM (2004) Wnt proteins in mammary development and cancer. J Mammary Gland Biol Neoplasia 9(2):119–131. doi:10.1023/B:JOMG.0000037157.94207.33
77. Lin TP, Guzman RC, Osborn RC, Thordarson G, Nandi S (1992) Role of endocrine, autocrine, and paracrine interactions in the development of mammary hyperplasia in Wnt-1 transgenic mice. Cancer Res 52(16):4413–4419
78. Gunther EJ, Moody SE, Belka GK, Hahn KT, Innocent N, Dugan KD et al (2003) Impact of p53 loss on reversal and recurrence of conditional Wnt-induced tumorigenesis. Genes Dev 17(4):488–501. doi:10.1101/gad.1051603
79. Geyer FC, Lacroix-Triki M, Savage K, Arnedos M, Lambros MB, MacKay A et al (2011) Beta-catenin pathway activation in breast cancer is associated with triple-negative phenotype but not with CTNNB1 mutation. Mod Pathol 24(2):209–231. doi:10.1038/modpathol.2010.205
80. Lin SY, Xia W, Wang JC, Kwong KY, Spohn B, Wen Y et al (2000) Beta-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. Proc Natl Acad Sci U S A 97(8):4262–4266. doi:10.1073/pnas.060025397
81. Rosenberg LH, Lafitte M, Quereda V, Grant W, Chen W, Bibian M et al (2015) Therapeutic targeting of casein kinase 1delta in breast cancer. Sci Transl Med 7(318):318ra202. doi:10.1126/scitranslmed.aac8773
82. Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, Ting DT et al (2013) Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science 339(6119):580–584. doi:10.1126/science.1228522
83. Guo W, Keckesova Z, Donaher JL, Shibue T, Tischler V, Reinhardt F et al (2012) Slug and Sox9 cooperatively determine the mammary stem cell state. Cell 148(5):1015–1028. doi:10.1016/j.cell.2012.02.008
84. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G et al (2008) The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 10(5):593–601. doi:10.1038/ncb1722
85. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C et al (2004) Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 117(7):927–939. doi:10.1016/j.cell.2004.06.006
86. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY et al (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133(4):704–715. doi:10.1016/j.cell.2008.03.027
87. Scheel C, Eaton EN, Li SH, Chaffer CL, Reinhardt F, Kah KJ et al (2011) Paracrine and autocrine signals induce and maintain mesenchymal and stem cell states in the breast. Cell 145(6):926–940. doi:10.1016/j.cell.2011.04.029
88. Memmi EM, Sanarico AG, Giacobbe A, Peschiaroli A, Frezza V, Cicalese A et al (2015) p63 sustains self-renewal of mammary cancer stem cells through regulation of sonic hedgehog signaling. Proc Natl Acad Sci U S A 112(11):3499–3504. doi:10.1073/pnas.1500762112
89. Cordenonsi M, Zanconato F, Azzolin L, Forcato M, Rosato A, Frasson C et al (2011) The hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. Cell 147(4):759–772. doi:10.1016/j.cell.2011.09.048
90. Bartucci M, Dattilo R, Moriconi C, Pagliuca A, Mottolese M, Federici G et al (2015) TAZ is required for metastatic activity and chemoresistance of breast cancer stem cells. Oncogene 34(6):681–690. doi:10.1038/onc.2014.5
91. Creighton CJ, Li X, Landis M, Dixon JM, Neumeister VM, Sjolund A et al (2009) Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. Proc Natl Acad Sci U S A 106(33):13820–13825. doi:10.1073/pnas.0905718106
92. Perou CM (2011) Molecular stratification of triple-negative breast cancers. Oncologist 16(Suppl 1):61–70. doi:10.1634/theoncologist.2011-S1-61
93. Luo M, Brooks M, Wicha MS (2015) Epithelial-mesenchymal plasticity of breast cancer stem cells: implications for metastasis and therapeutic resistance. Curr Pharm Des 21(10):1301–1310
94. Davis DG, Siddiqui MT, Oprea-Ilies G, Stevens K, Osunkoya AO, Cohen C et al (2016) GATA-3 and FOXA1 expression is useful to differentiate breast carcinoma from other carcinomas. Hum Pathol 47(1):26–31. doi:10.1016/j.humpath.2015.09.015
95. Kouros-Mehr H, Werb Z (2006) Candidate regulators of mammary branching morphogenesis identified by genome-wide transcript analysis. Dev Dyn 235(12):3404–3412. doi:10.1002/dvdy.20978

96. Asselin-Labat ML, Sutherland KD, Barker H, Thomas R, Shackleton M, Forrest NC et al (2007) Gata-3 is an essential regulator of mammary-gland morphogenesis and luminal-cell differentiation. Nat Cell Biol 9(2):201–209. doi:10.1038/ncb1530
97. Bernardo GM, Lozada KL, Miedler JD, Harburg G, Hewitt SC, Mosley JD et al (2010) FOXA1 is an essential determinant of ERalpha expression and mammary ductal morphogenesis. Development 137(12):2045–2054. doi:10.1242/dev.043299
98. Zaret KS, Carroll JS (2011) Pioneer transcription factors: establishing competence for gene expression. Genes Dev 25(21):2227–2241. doi:10.1101/gad.176826.111
99. Liu Z, Merkurjev D, Yang F, Li W, Oh S, Friedman MJ et al (2014) Enhancer activation requires trans-recruitment of a mega transcription factor complex. Cell 159(2):358–373. doi:10.1016/j.cell.2014.08.027
100. Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. Nature 490(7418):61–70. doi:10.1038/nature11412
101. Usary J, Llaca V, Karaca G, Presswala S, Karaca M, He X et al (2004) Mutation of GATA3 in human breast tumors. Oncogene 23(46):7669–7678. doi:10.1038/sj.onc.1207966
102. Adomas AB, Grimm SA, Malone C, Takaku M, Sims JK, Wade PA (2014) Breast tumor specific mutation in GATA3 affects physiological mechanisms regulating transcription factor turnover. BMC Cancer 14:278. doi:10.1186/1471-2407-14-278
103. Takaku M, Grimm SA, Wade PA (2015) GATA3 in breast cancer: tumor suppressor or oncogene? Gene Expr 16(4):163–168. doi:10.3727/105221615X14399878166113
104. Glubb DM, Maranian MJ, Michailidou K, Pooley KA, Meyer KB, Kar S et al (2015) Fine-scale mapping of the 5q11.2 breast cancer locus reveals at least three independent risk variants regulating MAP3K1. Am J Hum Genet 96(1):5–20. doi:10.1016/j.ajhg.2014.11.009
105. Kouros-Mehr H, Bechis SK, Slorach EM, Littlepage LE, Egeblad M, Ewald AJ et al (2008) GATA-3 links tumor differentiation and dissemination in a luminal breast cancer model. Cancer Cell 13(2):141–152. doi:10.1016/j.ccr.2008.01.011
106. Mazzarella L, Riva L, Luzi L, Ronchini C, Pelicci PG (2014) The genomic and epigenomic landscapes of AML. Semin Hematol 51(4):259–272. doi:10.1053/j.seminhematol.2014.08.007
107. Li B, Carey M, Workman JL (2007) The role of chromatin during transcription. Cell 128(4):707–719. doi:10.1016/j.cell.2007.01.015
108. Cai SF, Chen CW, Armstrong SA (2015) Drugging chromatin in cancer: recent advances and novel approaches. Mol Cell 60(4):561–570. doi:10.1016/j.molcel.2015.10.042
109. Ruthenburg AJ, Allis CD, Wysocka J (2007) Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. Mol Cell 25(1):15–30. doi:10.1016/j.molcel.2006.12.014
110. Fang F, Turcan S, Rimner A, Kaufman A, Giri D, Morris LG et al (2011) Breast cancer methylomes establish an epigenomic foundation for metastasis. Sci Transl Med 3(75):75ra25. doi:10.1126/scitranslmed.3001875
111. Stirzaker C, Zotenko E, Song JZ, Qu W, Nair SS, Locke WJ et al (2015) Methylome sequencing in triple-negative breast cancer reveals distinct methylation clusters with prognostic value. Nat Commun 6:5899. doi:10.1038/ncomms6899
112. Maruyama R, Choudhury S, Kowalczyk A, Bessarabova M, Beresford-Smith B, Conway T et al (2011) Epigenetic regulation of cell type-specific expression patterns in the human mammary epithelium. PLoS Genet 7(4):e1001369. doi:10.1371/journal.pgen.1001369
113. Azuara V, Perry P, Sauer S, Spivakov M, Jorgensen HF, John RM et al (2006) Chromatin signatures of pluripotent cell lines. Nat Cell Biol 8(5):532–538. doi:10.1038/ncb1403
114. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J et al (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125(2):315–326. doi:10.1016/j.cell.2006.02.041
115. Chaffer CL, Marjanovic ND, Lee T, Bell G, Kleer CG, Reinhardt F et al (2013) Poised chromatin at the ZEB1 promoter enables breast cancer cell plasticity and enhances tumorigenicity. Cell 154(1):61–74. doi:10.1016/j.cell.2013.06.005
116. Su Y, Subedee A, Bloushtain-Qimron N, Savova V, Krzystanek M, Li L et al (2015) Somatic cell fusions reveal extensive heterogeneity in basal-like breast cancer. Cell Rep 11(10):1549–1563. doi:10.1016/j.celrep.2015.05.011
117. Sighoko D, Liu J, Hou N, Gustafson P, Huo D (2014) Discordance in hormone receptor status among primary, metastatic, and second primary breast cancers: biological difference or misclassification? Oncologist 19(6):592–601. doi:10.1634/theoncologist.2013-0427
118. Liu S, Dontu G, Mantle ID, Patel S, Ahn NS, Jackson KW et al (2006) Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. Cancer Res 66(12):6063–6071. doi:10.1158/0008-5472.CAN-06-0054
119. Pietersen AM, Evers B, Prasad AA, Tanger E, Cornelissen-Steijger P, Jonkers J et al (2008) Bmi1 regulates stem cells and proliferation and differentiation of committed cells in mammary epithelium. Curr Biol 18(14):1094–1099. doi:10.1016/j.cub.2008.06.070
120. Wassef M, Rodilla V, Teissandier A, Zeitouni B, Gruel N, Sadacca B et al (2015) Impaired PRC2 activity promotes transcriptional instability and favors breast tumorigenesis. Genes Dev 29(24):2547–2562. doi:10.1101/gad.269522.115
121. Pal B, Bouras T, Shi W, Vaillant F, Sheridan JM, Fu N et al (2013) Global changes in the mammary epigenome are induced by hormonal cues and coordinated by Ezh2. Cell Rep 3(2):411–426. doi:10.1016/j.celrep.2012.12.020
122. Michalak EM, Nacerddine K, Pietersen A, Beuger V, Pawlitzky I, Cornelissen-Steijger P et al (2013) Polycomb group gene Ezh2 regulates mammary gland morphogenesis and maintains the luminal progenitor pool. Stem Cells 31(9):1910–1920. doi:10.1002/stem.1437
123. Bachmann IM, Halvorsen OJ, Collett K, Stefansson IM, Straume O, Haukaas SA et al (2006) EZH2 expression is associated with high proliferation rate and aggressive tumor subgroups in cutaneous melanoma and cancers of the endometrium, prostate, and breast. J Clin Oncol 24(2):268–273. doi:10.1200/JCO.2005.01.5180
124. Simon JA, Lange CA (2008) Roles of the EZH2 histone methyltransferase in cancer epigenetics. Mutat Res 647(1–2):21–29. doi:10.1016/j.mrfmmm.2008.07.010
125. Luense S, Denner P, Fernandez-Montalvan A, Hartung I, Husemann M, Stresemann C et al (2015) Quantification of histone H3 Lys27 trimethylation (H3K27me3) by high-throughput microscopy enables cellular large-scale screening for small-molecule EZH2 inhibitors. J Biomol Screen 20(2):190–201. doi:10.1177/1087057114559668
126. Curry E, Green I, Chapman-Rothe N, Shamsaei E, Kandil S, Cherblanc FL et al (2015) Dual EZH2 and EHMT2 histone methyltransferase inhibition increases biological efficacy in breast cancer cells. Clin Epigenetics 7(1):84. doi:10.1186/s13148-015-0118-9
127. Tan J, Yang X, Zhuang L, Jiang X, Chen W, Lee PL et al (2007) Pharmacologic disruption of polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. Genes Dev 21(9):1050–1063. doi:10.1101/gad.1524107

128. Vigushin DM, Ali S, Pace PE, Mirsaidi N, Ito K, Adcock I et al (2001) Trichostatin a is a histone deacetylase inhibitor with potent antitumor activity against breast cancer in vivo. Clin Cancer Res 7(4):971–976
129. Kawai H, Li H, Avraham S, Jiang S, Avraham HK (2003) Overexpression of histone deacetylase HDAC1 modulates breast cancer progression by negative regulation of estrogen receptor alpha. Int J Cancer 107(3):353–358. doi:10.1002/ijc.11403
130. Jang ER, Lim SJ, Lee ES, Jeong G, Kim TY, Bang YJ et al (2004) The histone deacetylase inhibitor trichostatin a sensitizes estrogen receptor alpha-negative breast cancer cells to tamoxifen. Oncogene 23(9):1724–1736. doi:10.1038/sj.onc.1207315
131. Hodges-Gallagher L, Valentine CD, Bader SE, Kushner PJ (2007) Inhibition of histone deacetylase enhances the anti-proliferative action of antiestrogens on breast cancer cells and blocks tamoxifen-induced proliferation of uterine cells. Breast Cancer Res Treat 105(3):297–309. doi:10.1007/s10549-006-9459-6
132. Thomas S, Thurn KT, Bicaku E, Marchion DC, Munster PN (2011) Addition of a histone deacetylase inhibitor redirects tamoxifen-treated breast cancer cells into apoptosis, which is opposed by the induction of autophagy. Breast Cancer Res Treat 130(2):437–447. doi:10.1007/s10549-011-1364-y
133. Yardley DA, Ismail-Khan RR, Melichar B, Lichinitser M, Munster PN, Klein PM et al (2013) Randomized phase II, double-blind, placebo-controlled study of exemestane with or without entinostat in postmenopausal women with locally recurrent or metastatic estrogen receptor-positive breast cancer progressing on treatment with a nonsteroidal aromatase inhibitor. J Clin Oncol 31(17):2128–2135. doi:10.1200/JCO.2012.43.7251
134. Zou MR, Cao J, Liu Z, Huh SJ, Polyak K, Yan Q (2014) Histone demethylase jumonji AT-rich interactive domain 1B (JARID1B) controls mammary gland development by regulating key developmental and lineage specification genes. J Biol Chem 289(25):17620–17633. doi:10.1074/jbc.M114.570853
135. Yamamoto S, Wu Z, Russnes HG, Takagi S, Peluffo G, Vaske C et al (2014) JARID1B is a luminal lineage-driving oncogene in breast cancer. Cancer Cell 25(6):762–777. doi:10.1016/j.ccr.2014.04.024
136. Whyte WA, Bilodeau S, Orlando DA, Hoke HA, Frampton GM, Foster CT et al (2012) Enhancer decommissioning by LSD1 during embryonic stem cell differentiation. Nature 482(7384):221–225. doi:10.1038/nature10805
137. Wang Y, Zhang H, Chen Y, Sun Y, Yang F, Yu W et al (2009) LSD1 is a subunit of the NuRD complex and targets the metastasis programs in breast cancer. Cell 138(4):660–672. doi:10.1016/j.cell.2009.05.050
138. Gu B, Watanabe K, Sun P, Fallahi M, Dai X (2013) Chromatin effector Pygo2 mediates Wnt-notch crosstalk to suppress luminal/alveolar potential of mammary stem and basal cells. Cell Stem Cell 13(1):48–61. doi:10.1016/j.stem.2013.04.012
139. Andrews PG, Lake BB, Popadiuk C, Kao KR (2007) Requirement of Pygopus 2 in breast cancer. Int J Oncol 30(2):357–363
140. Loven J, Hoke HA, Lin CY, Lau A, Orlando DA, Vakoc CR et al (2013) Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell 153(2):320–334. doi:10.1016/j.cell.2013.03.036
141. Shu S, Lin CY, He HH, Witwicki RM, Tabassum DP, Roberts JM et al (2016) Response and resistance to BET bromodomain inhibitors in triple-negative breast cancer. Nature. doi:10.1038/nature16508