Review

Elf5, hormones and cell fate

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Recent elucidation of the stem and progenitor cell hierarchies that operate during normal tissue and organ development has provided a foundation for the development of new insights into the disease process. These hierarchies are established by genetic mechanisms, which specify and determine cell fate and act as cell-clade gatekeepers, upon which all multicellular organisms depend for viability. Perturbation of this gatekeeper function characterizes developmentally based diseases, such as cancer. Here, the emerging gatekeeper and master regulatory roles of the ETS transcription factor Elf5 in several diverse developmental scenarios is reviewed, and how this function intersects with hormonal and growth factor mediated regulation of these processes is shown.

Cell hierarchies and the genesis of diversity in cancer phenotypes

Asymmetric stem cell division starts the production of new cells, which make several cell fate decisions as they move toward their completely differentiated state. Each decision creates a new lineage, further restricting the possible final phenotypes. The process of choosing between mutually exclusive cell fates starts with the preliminary nonbinding step of specification and becomes determined once the fate of the cell is irreversibly sealed. Specification involves *de novo* transcription factor expression and repression during which fate remains plastic, whereas determination involves the development of feedback loops to fix cell fate. Epigenetic mechanisms are involved in both specification and determination, and ensure stable inheritance of the fixed phenotype during subsequent cell divisions.

Transdifferentiation (where one differentiated cell type changes to become another differentiated cell type from an apparently mutually-exclusive lineage) or dedifferentiation (where differentiated cells regain pluripotency) occur in a few anomalous situations, such as metaplasia in response to irritation [1] or induced pluripotency by forced Yamanaka factor expression (see Glossary) [2]. Overwhelmingly, however, cell lineages act as inescapable clades providing the lineage stability on which multicellular organisms depend. Recent elucidation of the stem and progenitor cell hierarchies that establish these clades have provided a new view of carcinogenesis. The phenotypic differences that have long been observed in cancers from the same organ are now being attributed to different cells of origin within the stem and progenitor hierarchy. Fragments of normal developmental mechanisms may persist within tumors or may be hijacked by oncogenic lesions. Cancer cells also become adept at transdifferentiation between epithelial and mesenchymal phenotypes [3]. This fusion of developmental biology with carcinogenesis offers not only a better understanding of cancer but also new biomarkers to guide treatment decisions and new therapeutic strategies. This review focuses on the role of the ETS transcription factor Elf5 in cell fate decision making.

Elf5 structure and function

E74-like factor 5 (Elf5, Entrez Gene 2001, Ensembl ENSG00000135374, UniProtKB Q9UKW6) is an epithelial specific member of the ETS transcription factor family. It is

Glossary

Alveolar: located within an alveolus in the mammary gland, the basic spherical milk secreting units that are packaged together to form alveoli and are further aggregated to form the lobuloalveolar structures known in humans as the terminal ductal lobuloalveolar unit.
Basal: cellular position within the duct located at the basement membrane, without a luminal face. Note: many cells have both ductal basal and luminal faces resulting in confusion. Basal is used mainly to describe myoepithelial cells and cells that sort with them during flow cytometry. These terms are confusingly also used to describe patterns of gene expression that distinguish the intrinsic molecular subtypes of breast cancer.
Basal subtype: breast cancers which are generally estrogen receptor negative and are associated with poor prognosis.
Dedifferentiation: the process of reprogramming a differentiated cell to obtain pluripotency.
Determination: the process of choosing one fate over another. Mechanisms operate to end specification by fixing a decision, causing the loss of gene expression associated with the alternative fate and stabilizing the decision often via inheritable epigenetic mechanisms.
Intrinsic molecular subtypes: the types of breast cancer distinguished by their patterns of gene expression.
Loss of heterozygosity (LOH): loss of heterozygosity in cancer is the loss of a wild-type allele to expose the activity of a mutant allele, either by deletion of the region containing the wild-type allele or by genetic damage resulting in its replacement by a mutant allele. Seen as the loss of heterozygous markers in cancer, compared to somatic cells.
Luminal: cellular position within the mammary duct located at the face of the lumen.
Luminal subtype: breast cancers that are generally estrogen receptor positive.
Multipotency: the ability of a cell to contribute to multiple cell lineages, typically restricted to a single tissue.
Paracrine signaling: secretion by a cell of a factor that modulates a nearby cell.
Pluripotency: the ability of a cell to make all three germ layers but not placenta (e.g. induced pluripotent stem cells).
Specification: the process by which a cell fate decision is initiated by the *de novo* expression of specifying factors, giving the cell a choice of phenotypic fates that did not previously exist and which remains plastic. Often, the cell expresses aspects of both fates simultaneously.
Totipotency: the ability of a cell to make all cell types including placenta (e.g. an embryonic stem cell).
Transdifferentiation: the process by which a cell undergoes a phenotypic switch to assume the appearance of differentiated cells from a mutually exclusive clade. It is also called metaplasia. Epithelial to mesenchymal transition and apocrine metaplasia are examples observed in cells from the breast.

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Box 1. Elf5 directs lung morphogenesis; emergence of a putative role for Elf5 in mediating fibroblast growth factor regulated processes

The fibroblast growth factor (FGF) signaling pathway consists of four receptor genes that are alternatively spliced to produce at least seven distinct receptors. Multiple ligands exist that can be divided into families based on their homology and each family has a specific pattern of receptor specificity [64]. All four FGF receptor genes are expressed in the lung.

Deletion of Fgfr3 or Fgfr4, results in postnatal lung alveologenesis defects [65], whereas deletion of Fgfr2b or Fgf10 results in failed lung development during embryogenesis [66]. Blockade of pan fibroblast growth factor signaling with the chemical inhibitor SU5402 in cultures of embryonic lung inhibited branching morphogenesis. In addition, microarray profiling identified that Elf5 mRNA is downregulated under these conditions, whereas treatment with the hormones Fgf7 and Fgf10 strongly up-regulated Elf5 expression [67]. Fgf10 is produced by the lung mesenchyme and signals via Fgfr2b, to elicit branching morphogenesis of the epithelial pulmonary tree.

Elf5 regulates the expression of keratin 18 in pulmonary cells [68]. It has been observed that when Elf5 is expressed in lung epithelium during early development, branching is disrupted, cell proliferation is reduced and distal differentiation is inhibited, resulting in the formation of a dilated epithelium. Forced expression of the protein at later stages of development is without effect [69], leading to the conclusion that forced Elf5 expression prevents the normal spatial changes in native Elf5 expression, including the loss of its distal expression that is observed late in development, with consequential persistence of distal epithelial progenitor cells and failed cell differentiation, which in turn produces the previously mentioned defects.

Given the interaction of Fgfs with Elf5 in the embryo it is likely that Elf5 is generally involved in the interpretation of Fgf signaling [23].

located on human 11p13-15, a region subject to loss of heterozygosity (LOH) in cancer [4]. It contains two recognizable domains, a motif that interacts with a core TTCC DNA motif [5] carried by all ETS transcription factors and a pointed domain which is involved in protein–protein interactions. Elf5 is also known as ESE2 in humans where two isoforms exist (ESE2a and ESE2b) due to alternate transcriptional start sites localized in different exon 1s. Each isoform also shows alternative splicing of 1 or 2 exons to remove the pointed domain. Whether this transcript variety is due to rare transcriptional outputs or functionally relevant diversity remains to be elucidated. Elf5 is expressed during embryogenesis at several embryonic and neonatal sites including the hair follicle [6], epithelial exocrine glands [7], kidney [8], keratinocytes [9–11] prostate [12], lung (Box 1) and the mammary gland.

The first cell fate decision

Fertilization initiates rapid cell division to produce outside and inside cells contained within the zona pellucida of the early blastocyst (Figure 1a). Additional hypothesized polarizing influences include the ellipsoidal (not spherical) shape of the zona pellucida, the association of the second polar body with the inner cell mass and the nonsymmetrical division pattern of the first blastomeres, leading to the differential inheritance of cell surfaces that have experienced different extracellular contacts [13–15]. These polarizing processes initiate the first cell fate decision, which distinguishes the trophectoderm from the inner cell mass of the blastocyst, 4 days after fertilization (Figure 1a), to produce the separate developmental paths of the fetus and placenta. Specification of the trophectoderm becomes apparent from the 16 cell stage and although spatial organization is achieved by the early blastocyst stage, trophectoderm cell fate is not determined until the late blastocyst stage, when trophoblast cells can no longer become inner cell mass cells [16].

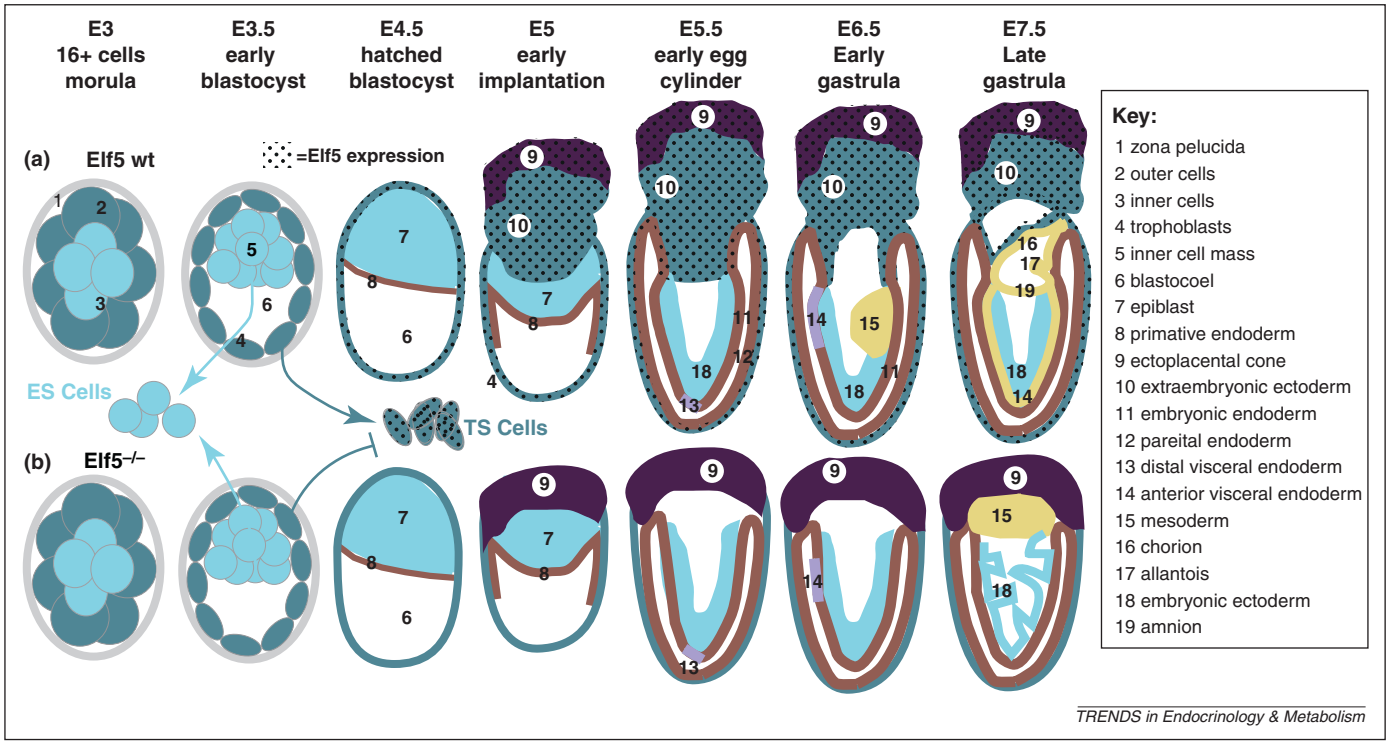

Figure 1. The role of Elf5 in determination of the trophectoderm. **(a)** shows the normal embryonic development from the 16+ cell stage at embryonic day 3 (E3) to late gastrulation at E7.5 days. **(b)** shows the aberrant development observed in Elf5$^{-/-}$ embryos. Elf5 expression pattern is shown as pointed shading and the key indicates the structures of the early embryo. Trophectoderm stem cells (TS) can be derived from wild-type (wt) blastocysts but not Elf5 null-mutant (Elf5$^{-/-}$) blastocysts, whereas embryonic stem cells (ES) can be derived from blastocysts of both genotypes.

Elf5 determines trophectoderm cell fate

Knockout of Elf5 in the mouse produces embryonic lethality [17,18]. Mendelian ratios diverge from normal at embryonic day 8.5 (E8.5), but developmental defects are seen from as early as E5 (Figure 1b). Elf5 knockout embryos are also smaller than wild type from E5 onward and do not show the extraembryonic–embryonic ectoderm constriction [17]. Staining for the homeobox protein Cdx2, a transcription factor that marks the extra embryonic ectoderm, can be observed in the trophectoderm of Elf5 knockout blastocysts at E4.5, but is lost in later stage embryos [17]. Staining for other markers of the extraembryonic ectoderm, such as Eomes (a transcriptional activator with a crucial role during development), fibroblast growth factor receptor 2 (Fgfr2), bone morphogenetic protein 4 (Bmp4), the enzymes furin and subtilisin-like proprotein convertase PACE4 (Spc4) is absent, indicating that the extraembryonic ectoderm does not form from the trophectoderm [17]. By contrast, the ectoplacental cone does form from the trophectoderm, as do the giant trophoblast cells at the exterior of the distal and anterior visceral endoderm (Figure 1b). Mesoderm does not form and gastrulation does not commence at E6.5 [17]. Half of the Elf5$^{-/-}$ embryos show late formation of mesoderm, but it is located beneath the ectoplacental cone, rather than invading the region between endoderm and ectoderm [17]. A chorion is never observed and consequently the Elf5 embryos die from nutrient restriction from E8 [17].

Totipotency of the cells of the inner cell mass depends on *de novo* expression of the transcription factors octamer-binding transcription factor 4 (Oct4), the homeobox protein Nanog, Sal-like protein 4 (Sall4) and SRY (sex determining region Y)-box 2 (Sox2) (Figure 2). These factors form a self reinforcing transcriptional circuit specifying totipotency [16], but it can be overcome. Forced expression of Cdx2 and Eomes, together with culture in fibroblast growth factor containing trophectoderm stem (TS) media, causes the embryonic stem (ES) cells to become more restricted TS cells, despite the continued expression of the totipotency

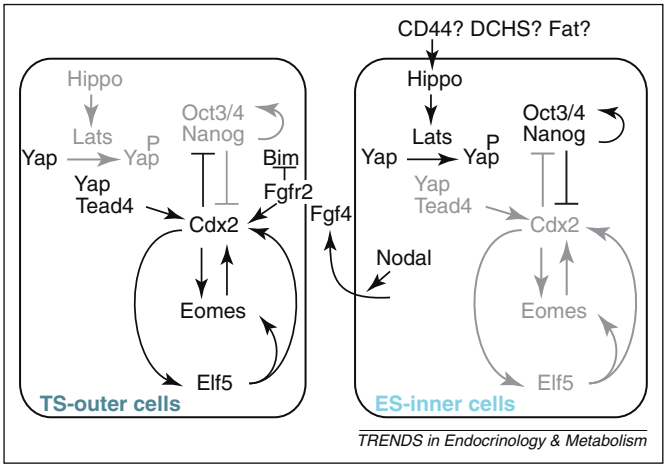

Figure 2. The transcriptional network that determines embryonic cell fate. Cell fate decisions made within the early blastocyst may be initiated by spatial cues delivered via the Hippo pathway to the inner cells (light blue in Figure 1) This signal reinforces expression of Oct3/4 and Nanog by suppression of Cdx2/Elf5, and sends a paracrine signal via secretion of Fgfs that reinforces the opposite actions in the outer cells. By this mechanism the fates of these cells diverge to produce the embryo and placenta. Black type and arrows show active pathways. Grey arrows and type show inactive or absent pathways.

genes [19]. Cdx2 is under the positive control of Fgf4 (via the Fgfr2 receptor and paracrine signaling) [16] and the Hippo pathway. The Hippo pathway senses external spatial cues and responds via the Lats protein kinase [13], which phosphorylates Yes associated protein (Yap), preventing its association with Tead4 and induction of Cdx2 (Figure 2). From the 16-cell stage these pathways drive Cdx2 and Eomes expression, especially in the most outer cells, conferring trophectoderm specification. Although the Hippo pathway acts to sense external positional cues, Fgf4 is secreted by the ES cells of the epiblast and signals via the Fgfr2 receptor on TS cells, activating the Src/Ras/Erk pathway to maintain Cdx2 expression and to prevent apoptosis via the pro-apoptotic protein Bim (Figure 2) [20].

ES cells but not TS cells can be isolated from Elf5 knockout embryos, indicating that Elf5 is essential for the expansion of the trophoblast lineage [17]. ES cells isolated from mice that have been made methylation deficient by the deletion of DNA methyltransferases, are unable to maintain their pluripotent identity when they are cultured in fibroblast growth factor containing TS medium and differentiate to become trophoblast derivatives [21]. A genome wide screen using immunoprecipitation of methylated DNA and searching for genes with promoters that showed methylation in ES, as compared to TS cells, identified Elf5 as a gene with promoter methylation in ES cells [21].

Indeed, in the mouse, methylation and consequential silencing of the Elf5 promoter, or knockout of Elf5, makes determination of TS cell fate impossible, since Elf5 is not available to participate in a positive feedback loop with Cdx2 (Figure 2). As a result, the cells of the trophectoderm that are specified and are formed at the early blastocyst stage cannot be determined and so following implantation they differentiate [21,22] to form the ectoplacental cone and giant trophoblast cells, with the result that no extraembryonic ectoderm forms [17]. However, the role of methylation of the Elf5 promoter in fixing this positive feedback loop was challenged by the observation that deletion of the methylated regions 1 and 2 of the Elf5 promoter has no effect on Elf5 expression in trophectoderm [23]. Instead trophectoderm-specific enhancer regions were defined and it was hypothesized that they primarily drive Elf5 expression [23]. This deletion, however, did not remove methylated regions 3 and 4 of the Elf5 promoter, regions that show altered patterns of methylation that are correlated with Elf5 expression in the mammary gland [24]. A more definitive analysis of the methylation status of the Elf5 promoter in ES and TS cell, and a more complete deletion mutagenesis of the Elf5 promoter, are required to answer this question.

The extent to which Elf5 operates to specify trophectoderm has also been examined in humans, cattle and pigs. In cattle, Elf5 is localized to the epiblast not the trophectoderm of blastocysts [23], but in pigs Elf5 is expressed only by the trophectoderm [25]. Like all ungulates, neither species forms an extraembryonic ectoderm [23]. Homologous sequences to the mouse enhancers [23] are not present in the bovine Elf5 promoter and no activity of the bovine Elf5 promoter is seen within the mouse trophectoderm [23]. Thus the mouse has evolved trophoblastic-specific enhancers

within the Elf5 gene, associated with the evolutionary development of the extraembryonic ectoderm [23]. In humans the situation resembles that of the mouse. Elf5 is hypomethylated and expressed in the cytotrophoblasts of the placenta and it establishes a similar regulatory network with Cdx2 and Eomes [26]. A putative TS cell population expresses both Elf5 and Cdx2 [26]. The Elf5 promoter is hypermethylated in ES cells and this is correlated with absence of Elf5 expression in ES cells and ES cell derivatives, which in humans can contribute to cells within the trophoblast lineage [26].

The hormonal control of mammary gland development

Five pairs of mammary placodes form at E11 in the mouse [27]. Under the control of fibroblast growth factors and other growth factors [28], around E15, the mammary primordium penetrates the mesenchyme to invade the underlying primordial mammary fat pad [27]. During subsequent gestational development, the multipotent mammary stem cell gives rise to the myoepithelial stem cell and the epithelial stem cell, providing the origin of the two major cell types of the postnatal gland. It appears at present that the multipotent stem cell persists within the mammary ducts, but ceases to provide a cell flux for further development, except in exceptional circumstances such as during wound recovery or following mammary epithelial transplantation [29]. At puberty, while under the influence of estrogen action on the epithelial cell estrogen receptor (ER) [30,31] and growth hormone action [32], the primordial duct forms a terminal end bud that invades and bifurcates throughout the mammary fat pad to produce a branched ductal structure. Ductal cellular architecture (Figure 3a) comprises a hollow lumen formed by a single layer of epithelial cells which are sheathed by a dense basement membrane containing myoepithelial cells along the inner surface and fibroblasts scattered along the outer surface, which interfaces with the adipocytes of the mammary fat pad.

Following puberty, progesterone acts via the epithelial progesterone receptor (PR) and at the head of a paracrine network to induce stem cell division [33–35] and the sprouting of further branches from the ductal tree, which together with prolactin causes the formation of small alveolar structures at the ductal termini [36–38]. The increase in these hormonal signals during pregnancy

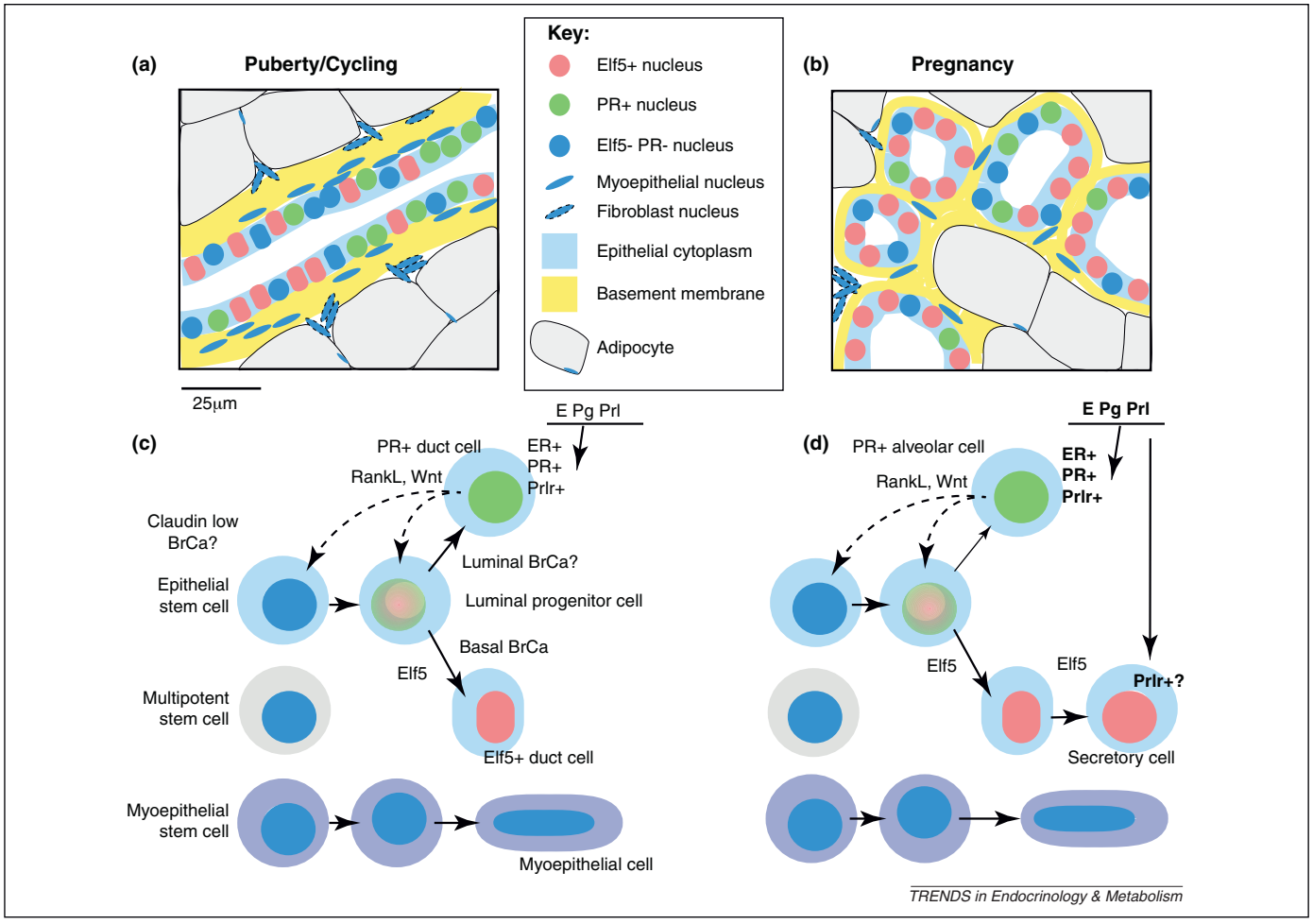

Figure 3. Elf5 in the mammary gland. Postnatal mammary development comprises two major events: **(a)** the formation of ducts during puberty and **(b)** the formation of additional milk-secreting alveoli during pregnancy. The nuclear pattern of Elf5 expression relative to progesterone receptor (PR) expression is shown at both developmental stages (see key). This architecture is produced by the activity of a stem and progenitor cell hierarchy illustrated in **(c)** and **(d)**. Hormonal signaling received by the hormone receptor positive ductal and alveolar cells ('sensor cells') signal cell via paracrine mechanisms (broken arrows), to initiate rounds of stem and progenitor cell divisions (unbroken arrows). It is proposed that the fates of these progenitors are directed by competition between sex-steroid hormone receptors and Elf5, so that during pregnancy, most of the stem cell progeny generated in response to hormone signals via Rankl and other paracrine mediators differentiate in response to Elf5, to establish the secretory cell lineage and alveolargenesis **(d)**. The competition between sex steroid signaling and Elf5 for this cell fate decision may also influence the subtype of breast cancer produced by the luminal progenitor cell. It is most likely that secretory cells also express prolactin receptors to allow milk protein synthesis in response to prolactin.

forces further branching and massive elaboration of the alveolar structures to produce the milk secreting lobuloalveolar units that occupy the entire mammary fat pad at term (Figure 3b). These comprise spherically arranged epithelial cells forming an alveolus with a lumen that drains to the ductal network and sheathed by a basement membrane. Sparse myoepithelial cells form a net-like structure around the alveoli. The loss of progesterone and the increase in prolactin during the immediate postpartum period initiates lactation. Following weaning and consequential loss of prolactin secretion, the gland involutes and returns to a developmental state that is very similar to the nulliparous gland. A fourth developmental stage can occur and this is the onset of hyperplastic lesions. The lesions can become cancer contained within the ducts and then progress to become locally invasive and metastatic disease. The hormones estrogen, prolactin and progesterone are also implicated in the carcinogenic process [34,39,40].

Prolactin regulates Elf5 action in mammary gland development

Elf5 is a prolactin regulated gene in the mammary gland. In prolactin receptor null mammary gland, Elf5 expression is much reduced, but prolactin receptor expression is unaltered in Elf5 knockout mammary gland, indicating that Elf5 is regulated by prolactin and the prolactin receptor [18,41,42]. In Scp2 mammary epithelial cells, Elf5 expression rises in response to prolactin treatment [41] and forced reexpression of Elf5 can rescue the failure of lobuloalveolar development that is observed in the prolactin receptor null mammary gland [41]. Choi and colleagues demonstrated that Elf5 null mammary glands have reduced Stat5 expression and phosphorylation [42]. This result implies that Elf5 acts upstream of Stat5 signaling and accordingly, Elf5 was shown to bind to the Stat5 promoter [42]. The signaling mechanisms by which prolactin activates Elf5 expression, however, remain to be determined.

It is possible that other hormones, especially those which cooperate with prolactin, may also regulate Elf5 action in the mammary gland. Recent reports have identified Elf5 as an insulin regulated gene in bovine and murine mammary gland explants [43,44] and progesterone has been shown to induce Elf5 expression in T47D cells, where Elf5 modulates the anti-proliferative effects of progesterone, in a feedback loop [45]. Mammary glands carrying null mutations for prolactin receptor, PR or Elf5 exhibit nearly identical defects of failed lobuloalveolar development. This similarity suggests that these hormones comprise a regulatory network pivotal to mammary development. Interactions between prolactin and progesterone have been described at the level of crossregulation of their receptors [46]. There is significant overlap in the transcriptional targets of progesterone and prolactin, as assessed by microarray analysis [47]. However, the molecular mechanisms that integrate prolactin and progesterone signaling pathways remain to be identified.

Elf5 in mammary development

In virgin mice Elf5 is expressed in the nucleus of approximately half of the luminal epithelial cells of the mammary gland and predominantly those of columnar appearance (Figure 3a) [24]. This pattern is mutually exclusive with ER [48], which is observed almost exclusively in round cells (Figure 3a). Elf5 transcription is silenced in the myoepithelial population [41], due to methylation of its promoter [24]. Elf5 expression in the mammary gland increases dramatically during pregnancy and is now seen in predominantly round cell nuclei of the newly formed alveoli (Figure 3b), whereas ER/PR positive cells become rare with generally just one or two per alveolus. Elf5 expression remains high during lactation and returns to baseline levels after involution [18,41].

Forced Elf5 expression in virgin mice, using an inducible mammary-specific transgene [48], results in alveolargenesis at ductal termini, similar to those illustrated in Figure 3b, although the extensive elaboration of these structures seen in pregnancy is not observed. Importantly, milk protein production is observed in the Elf5-induced alveoli, demonstrating functional differentiation. Induction of Elf5 expression in mid-pregnant mice also caused increased milk protein production [48]. The ability of Elf5 to induce milk protein expression in virgin mice is consistent with the observation that Elf5 binds directly to sites in the whey acidic protein (WAP) promoter and induces WAP expression in mammary cells [49]. Elf5 must also induce cell differentiation because expression of non-Elf5 target genes such as the caseins are also increased [48].

The effects of loss of Elf5 have been studied in heterozygous [18] and homozygous Elf5 knockout mice [48,50] and a mammary specific Elf5 knockout mouse has also been generated [42]. These studies collectively demonstrate that loss of Elf5 has no effect on ductal elongation during pubertal mammary development, but causes impairment of lobuloalveolargenesis during pregnancy. Elf5-deficient mammary glands display failed alveolar morphogenesis [48], which is characterized by the absence of mature lobuloalveolar structures, the inability to express milk proteins and nurse young [42], a sustained expression of ER and PR, a decrease in cellular proliferation [48] and other characteristics of virgin epithelium [42]. As evidenced from transplantation studies, these effects of Elf5 loss are epithelial cell autonomous [18,48].

Elf5, Stat5 and mammary stem and progenitor cells

Elf5 is not expressed in the stem cell enriched population of normal mammary glands, but is expressed in the luminal progenitor population [51] and in mature luminal cells (Figure 3c) [48]. In virgin Elf5 null mammary glands, there is no difference in the number or self-renewing potential of mammary stem cells, nor is there any change in the proportion of luminal progenitor cells [48]. During pregnancy, however, luminal progenitor cells accumulate in Elf5 null transplants and in $Elf5^{+/-}$ mammary glands, indicating that Elf5 is required for the differentiation of these cells. Consistent with this hypothesis, forced expression of Elf5 in virgin mice results in erosion of the luminal progenitor population which is accompanied by a decrease in the colony forming ability of these cells [48]. Notably Elf5 shows high expression in the luminal progenitor population [52,53]. Together these data indicate that Elf5 regulates the number and activity of luminal progenitor cells in the mammary gland

(Figure 3c). During pregnancy Elf5 transcriptional activity in the luminal progenitor population forces a cell fate decision that directs the cellular flux generated by divisions of the stem cell population toward the secretory lineage (Figure 3d). Stem cell division is initiated by progesterone and prolactin via a paracrine Rankl and other signals, and this mechanism also plays a role in hormonal carcinogenesis [34].

The position of Elf5 within the mammary cell hierarchy has been further defined by the study of Stat5 deficient mammary glands [51]. Despite having a similar morphology to Elf5 null mammary glands (normal development during puberty and failed alveolargenesis during pregnancy), Stat5 deficient mammary glands are not characterized by an accumulation of luminal progenitor cells during pregnancy. Rather, these glands display reduced proportions of luminal progenitor cells in virgin animals, suggesting that Stat5a is important for the generation of alveolar progenitor cells from stem cells. Consistent with this idea, Stat5a (unlike Elf5) is expressed in the mammary epithelial stem cell population and expression of Elf5 in the luminal progenitor population is dependent upon Stat5. Alone these results place Elf5 downstream of Stat5 in the mammary cell hierarchy, but as discussed above, Elf5 occupies the Stat5 promoter [42], which indicates the presence of a more complex genetic regulatory network underlying the control of cellular flux through the progenitor cell hierarchy by Elf5 and Stat5 [54]. Elucidation of this regulatory network will require further investigation.

Concluding remarks

A common and striking consequence of experimental modulation of Elf5 expression in the blastocyst, lung and mammary gland is aberrant cell fate decision making. In the blastocyst, lung and possibly the mammary primordia, these decisions are regulated to a large extent by fibroblast growth factors, suggesting that Elf5 may be required to interpret signals via the Fgf receptor signaling pathway [23]. Additional epigenetic mechanisms restrict Elf5 expression to the trophoblast lineage in the embryo and the epithelial lineages within the mammary gland, and presumably throughout the organism. In the mature mammary gland, Elf5 exerts much of the transcriptional effect of prolactin and progesterone, and by integrating these signals acts as a master regulator of alveolar development [55]. Cell fate decisions control the output of the stem and progenitor cell hierarchy and are crucial for tissue homeostasis. In the mammary gland, the stem and progenitor cell hierarchy has been hypothesized to explain much of the phenotypic diversity [56] seen in breast cancer [57]. The intrinsic molecular subtypes may originate from different stem and progenitor cells, with the claudin-low group hypothesized to come from a stem cell precursor [58], the basal subtype from the luminal progenitor cell [59] and the luminal subtype likely to also originate from this progenitor (Figure 3c). Since Elf5 is a key modulator of cell fate decisions taken by the luminal progenitor cell it is highly likely that it plays a role in the determination of breast cancer phenotype and subtype. Elf5 and other ETS factors are expressed by breast cancers [4,60–62]. By extension it is also likely that similar roles exist for Elf5 in cancers originating in the other epithelia where it is expressed. Much remains to be discovered regarding Elf5 action. Its binding partners remain elusive, but almost certainly exist as this mechanism is well conserved among ETS transcription factors. Defining the suite of direct transcriptional targets of Elf5 [63] will shed light on the genomic regulatory mechanism that it commands. Finally, uncovering more of the upstream regulators of Elf5 expression, both transcriptional and epigenetic, will link Elf5 to other developmental mechanisms and together with the preceding missing pieces of this puzzle offer an understanding of Elf5 action, which may be of great therapeutic use in breast and other cancers.

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