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Delivering the message: epimorphin and mammary epithelial morphogenesis[★]

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Abstract

The mammary gland consists of a highly branched tubular epithelium surrounded by a complex mesenchymal stroma. Epimorphin is an extracellular protein that is expressed by mammary mesenchymal cells that directs epithelial morphogenesis. Depending upon the context of presentation – polar versus apolar – epimorphin can selectively direct two key processes of tubulogenesis: branching morphogenesis (processes involved in tubule initiation and extension) and luminal morphogenesis (required for enlargement of tubule caliber). Here, we outline the fundamentals of mammary gland development and describe the function of epimorphin in these processes. We conclude with a review of recent studies that suggest similar morphogenic roles for epimorphin in other glandular organs.

The mammary gland has long been an object of scientific scrutiny but the past three decades have witnessed an ever increasing interest in the processes involved in its development [1]. These studies have led to an appreciation of the relationship between organization and function in the breast, and have also provided key insights into the development of many other tissues and organs. Here, we discuss how investigations of the role of epimorphin in the morphogenesis of the mammary epithelium are shedding light on general mechanisms of epithelial morphogenesis.

The fully differentiated mammary gland consists of a system of branching ducts that collect the milk produced by alveoli at the end of the ducts and transport it to the nipple. Development of the mammary gland is initiated in the embryo (Fig. 1) with formation of the mammary placodes, dome-shaped structures that contain epithelial and mesenchymal cell layers [2]. Subsequent development of the primitive branched anlage, the precursor of the mammary gland epithelium, is controlled in a largely organ-autonomous fashion through close-range interactions with the mesenchymal stroma that surrounds the epithelium and contains fibroblasts, adipocytes, immune effector cells and cells of the vascular system. Many of the morphogenic signals are initiated in the mesenchymal stroma, as demonstrated by the ability of mammary stroma to direct patterns of mammary epithelial differentiation when recombined with tissue from the salivary gland or dorsal skin [3].

In contrast to prenatal development, systemic mammogenic hormones control most aspects of postnatal development: ductal elongation, side-branching, alveolar bud formation and functional differentiation of secretory lobuloalveoli [3]. At sexual maturity, the ductal system

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has grown to the limits of the fat pad and ductal tree complexity is further increased by secondary and tertiary branching. The central lumen extends in concert with the developing ducts and, although this appears to depend upon apoptosis of the central luminal cells [4], recent studies with cultured human cell lines suggest that lumina can still develop even if some apoptotic pathways are blocked [5].

The fact that most mammary gland development occurs postnatally allows for tissue recombination experiments in which mammary epithelium from one mouse is transplanted into the fat pad stroma of a mouse that has been cleared of its endogenous epithelium. More sophisticated variations of these experiments involve co-culture in the kidney capsule of epithelium from one mouse with stroma from another. Such techniques allow for elegant dissection of signaling pathways and have provided much information about the hormones that control mammary gland development [6]. For example, ductal elongation during puberty requires estrogen and growth hormone, whereas ductal branching and alveolar budding are also influenced by progesterone, prolactin and thyroid hormone [7]. Of these, estrogen, progesterone, prolactin and growth hormone can mediate effects on epithelial morphogenesis through binding to mesenchymal receptors [7], providing evidence of hormone-initiated, heterotypic signaling processes even if the signaling effector is unknown. For example, estrogen can stimulate epithelial ductal outgrowth through mesenchymal receptors [8], although the secondary signal has not been identified. With the exception of insulin-like growth factor, which acts as the effector of growth hormone [9], regulation of the mesenchyme-epithelium signals initiated by hormonal signaling are poorly understood. Signaling molecules that have been intensively investigated include hepatocyte growth factor (HGF) and transforming growth factor β (TGF β) [10,11]; here, we focus on epimorphin, a less-well-known factor that is now known to be important for mammary epithelial morphogenesis [12,13].

The double-layered tube: structural organization and developmental morphogenesis of the mammary gland

The branching ducts and terminal alveoli in the mammary gland are largely elaborated from a double-layered epithelial tube (Fig. 2). The inner layer is composed of luminal epithelial cells, bound into a continuous surface by a combination of tight junctions, desmosomes and E-cadherin-mediated adherens junctions. The outer layer is composed of myoepithelial cells, which play an essential role in glandular morphogenesis and are required for expelling milk during lactation. The double-layered epithelial structure forms in response to differential adhesion of luminal and myoepithelial cells, and the presentation of laminin-1 by the myoepithelial cells to the luminal epithelial cells [14,15].

The double-layered epithelium is surrounded by the basement membrane (BM), a specialized extracellular matrix (ECM) that provides structural support and contextual information to the underlying cells [16]. Outside the BM, the mammary stroma contains fibroblasts, adipocytes, blood vessels and immune cells, all embedded within a fibrous ECM [17]. The tight relationship between tissue structure and developmental morphogenesis is demonstrated by transgenic mouse models in which targeted disruption of the former leads to substantial alterations in the latter. For example, increased branching, precocious development of lobuloalveoli and milk protein expression in the virgin stage were seen in mice that express either the matrix metalloproteinase stromelysin-1 (SL-1/MMP-3) [18,19] or a truncated, nonfunctional form of E-cadherin [20] in the mammary epithelium. Developmental alterations during pregnancy, lactation or involution stages have also been observed with other alterations of tissue structure factors [6,21].

So, how does the double-layered tube elaborate into the structurally complex mammary gland? Development of all branched epithelial tissues has been shown to involve two principal

morphogenic activities [22] – branching morphogenesis for tubule initiation and extension, and luminal morphogenesis for formation of secretory acini. The mechanisms involved in regulation of luminal diameter of mammary ducts are largely unknown but might involve a combination of cell division and the secretion of fluid into the lumina [23]. Studies of genes involved in polycystic kidney disease have identified several fluid transporters and have suggested the existence of force-activated mechanisms that sense luminal size, although whether such mechanisms exist in the mammary gland is unknown. Control of branching morphogenesis has been more intensively studied [24]. Mammary gland branching morphogenesis can be divided into two distinct processes [21]: primary branches form from bifurcation of the highly proliferative advancing terminal end buds; secondary and tertiary branches sprout from mature ducts and require the dissolution of a continuous epithelial sheet concomitant with degradation and invasion of the surrounding ECM (Fig. 3). Both types of branching morphogenesis depend upon epithelium-mesenchyme signaling because, as the epithelium branches, the stroma must also undergo remodeling to make room for and to provide support to the developing epithelial tubes [25].

The plethora of interrelated signals controlling these processes *in vivo* has prompted the development of culture systems in which a subset of morphogenic processes can be isolated and studied. Several assays have been developed in which cultured mammary epithelial cells or isolated mammary organoids are grown in three-dimensional (3D) gels of reconstituted ECM material; these assays are particularly useful for identifying specific signaling molecules and studying the relevant signal transduction processes [26]. Several growth factors have been found to mediate branching morphogenesis of mammary epithelial cells in 3D collagen gels. In this regard, HGF has been a particular target of study [10], although epidermal growth factor (EGF), keratinocyte growth factor (KGF) and fibroblast growth factor 1 (FGF-1) can all induce mammary cells to branch [27]. Why so many different growth factors should mediate such similar morphogenic activities can be understood if the growth factor signaling acts primarily as a proliferative engine at the appropriate time whereas one or more separate morphogenic signals control the structural development. We propose that epimorphin can fulfill one or more of these morphogenic functions.

Context matters: epimorphin as a multifunctional morphogen

The role of epimorphin as an extracellular morphogen was originally identified in studies of lung branching morphogenesis [28,29]. The same molecule was subsequently identified as syntaxin-2, a member of the syntaxin family of vesicle fusion proteins [30,31] [epimorphin/syntaxin-2 appears to be unique in this regard because, so far, no other syntaxin family member is known to share this dual topology (see Box 1 for additional details on the relationship between epimorphin and syntaxins)]. Lung organ cultures, which contain both epithelial and mesenchymal cells, can branch in 3D collagen if recombinant growth factors are provided, but this activity is completely blocked by the addition of anti-epimorphin antibodies [28]. Since that initial discovery, epimorphin has been found to play a role in morphogenesis of many tissues (Table 1), although it is the mammary gland in which the action of epimorphin has been most clearly defined. In the mammary gland, epimorphin expression reflects its role in mesenchyme-to-epithelium signaling: in tissue sections of postnatal mouse mammary glands, epimorphin is expressed within the stroma and around the epithelial ducts, whereas staining of isolated primary cells show epimorphin expression in fibroblasts and myoepithelial cells, but not in luminal epithelial cells [12]. During lactation, epimorphin can also be found around luminal epithelial cells, in the ductal lumina and in the milk [32] (Box 2). As with lung organ cultures, branching morphogenesis can be induced in ‘tissue organoids’ isolated from the mammary gland, if the organoids are cultured in 3D collagen and treated with EGF, HGF, FGF-1 or KGF; also, as with the lung organ cultures, growth-factor-induced branching could be inhibited by the addition of anti-epimorphin antibodies [27].

Box 1**Epimorphin as a member of the syntaxin family of proteins**

The targeting and fusion of membranous organelles in eukaryotic cells is mediated by a highly conserved mechanism [65] (Fig. 1a). Exocytosis is a variation of this process in which a cytosolic vesicle fuses with the plasma membrane, leading to release of contents from the inside of the vesicle to the outside of the cell. Exocytosis has been most extensively studied in chemical synaptic transmission, which is mediated by the interaction of three prototypic molecules: synaptobrevin/VAMP (vesicle-associated membrane protein), SNAP-25 (25 kDa synaptosome-associated protein) and syntaxin-1. Extensive investigations using this model have defined a mechanism in which a ternary complex of these proteins is sufficient to trigger membrane fusion [65].

Although epimorphin was originally identified as a morphogen through the function-blocking ability of an anti-epimorphin monoclonal antibody, the same molecule was subsequently identified as syntaxin-2, a member of the highly conserved syntaxin protein family [30,31]. Later investigations have supported an initial supposition [31,66] that epimorphin/syntaxin-2 has distinct functions depending on its membrane topology. When localized to the extracellular face of the cell surface membrane, epimorphin functions in morphogenesis; when localized to the cytoplasmic face, syntaxin-2 has been shown to mediate degranulation of platelets and pancreatic acinar cells [67–71], and the acrosome reaction in spermatozoa [72–74]. This duality of function has complicated investigations in other organs. For example, epimorphin/-syntaxin-2 shows tissue-specific distribution in rat kidney [75] and distinct patterns of localization in polarized Madin–Darby canine kidney cells [51,76], although no specific function for syntaxin-2 in molecular exocytosis has yet been described in the kidney. At present, it is unclear how the relative topological orientation of epimorphin/syntaxin-2 is controlled (Box 2); identification of this regulatory mechanism will doubtless shed additional light on how the same molecule in different spatial contexts can behave so differently. It is an important consideration, however, that although epimorphin (extracellular) and syntaxin-2 (intracellular) are functionally distinct molecules, they might share common mechanisms, as the organization of glandular epithelia and polarized secretion of proteins are highly interdependent activities [77]. Functional analyses of epimorphin [12] (D. Radisky and M.J. Bissell, unpublished) and of syntaxins [78–80] have identified distinct regions of these molecules as essential for their respective mechanisms (Fig. 1b), and this will probably provide the means for dissecting the distinct morphogenic role of epimorphin from the vesicle fusion role of syntaxin-2. A key player still unidentified is the epimorphin receptor(s) on the surface of the target epithelial cells; discovery of this molecule is now a matter of primary importance.

Box 2**Extracellular localization of epimorphin/syntaxin-2**

Epimorphin/syntaxin-2 has no exocytosis-targeting signal sequence, even though it is localized to the extracellular face of the plasma membrane and can signal for morphogenesis [12]. As such, epimorphin appears to be a member of a category of mammalian proteins that cross membranes by non-classical mechanisms (i.e. not involving transit through the endoplasmic reticulum and Golgi apparatus). The best characterized examples of this category in mammalian systems are fibroblast growth factors 1 and 2 (FGF1 and FGF2) [82,83], and interleukin-1 β [84], which also lack signal sequences, although proteins that cross membranes by non-classical mechanisms are also found in simple eukaryotes (e.g. *Dictyostelium* [85] and yeast [86]), bacteria [87] and viruses [88].

Elucidation of the mechanisms of non-classical protein secretion has proved to be challenging, although it is clear that these processes are highly regulated [89–94]. One complicating factor in these investigations is the disparate functions fulfilled by these proteins. Some are active only outside the cell (e.g. FGF1, FGF2, IL-1 β), whereas others appear to have similar functions on both sides of the plasma membrane (e.g. sphingosine-1 kinase [95]). By contrast, epimorphin/syntaxin-2 appears to have completely distinct functions depending upon its membrane topology: facing inside, syntaxin-2 mediates membrane fusion; facing outside, epimorphin acts as a morphogen. As such, epimorphin/syntaxin-2 is an example of a subcategory of proteins that cross membranes by non-classical mechanisms and that also have clearly distinct extracellular and intracellular functions (Table I). For some of these, the extracellular and intracellular functions appear to be related: intracellular thioredoxin acts as an oxidoreductase [96], whereas extracellular thioredoxin is an inflammatory cytokine activated by conditions of oxidative stress [97]; the action of the chromatin protein HMGB1 as a proinflammatory signal might have derived from the presence of extracellular nuclear proteins following cellular apoptosis at sites of viral or bacterial infection [98]. For epimorphin/syntaxin-2, the functional relationship between the two proteins is less clear. The coiled-coil domains of syntaxin-2 might have been adapted as generic protein–protein interaction domains, in which case the mechanism by which epimorphin mediates morphogenesis might be unrelated to the well-studied mechanism by which syntaxin proteins mediate membrane fusion (Box 1).

In addition to luminal epithelial cells, primary mammary organoids also contain myoepithelial and mesenchymal cells, as well as a basement membrane [27]. Such heterogeneous models show considerable complexity with multiple overlapping interactions so, to dissect the specific downstream consequences of epimorphin signaling effectively, parallel investigations have used cultures of mammary luminal epithelial cell lines instead [33]. Evaluation of several cell lines showed that most expressed epimorphin, whereas no epimorphin expression was detectable in others. However, for both types of cell lines, epimorphin activity was required for morphogenesis. Growth-factor-induced branching of epimorphin-expressing cell lines could be inhibited with antibodies against both epimorphin and the particular exogenous growth factor used but not with antibodies against any other growth factor (i.e. HGF-induced branching could be inhibited by anti-HGF antibodies but EGF-induced branching could not) [12]. These results demonstrated that epimorphin was not acting by inducing the expression of alternative growth factors. For non-epimorphin-expressing luminal epithelial cell lines, branching required the addition of both exogenous epimorphin and any one of several different growth factors.

Experiments using recombinant epimorphin revealed its potential to mediate dramatically different morphogenic processes depending upon orientation of presentation [12]. Presented in a polar fashion to the basal surface of cell clusters, epimorphin stimulated branching morphogenesis; presented in an apolar fashion around all surfaces of the cells in the clusters, epimorphin instead stimulated the formation of cystic structures with large central lumen (Fig. 4). For both types of morphogenesis, the morphogenic signal was provided by epimorphin, whereas the extent of growth (branch length elongation or increase in luminal diameter) was proportional to the proliferative activity induced by the particular growth factor [12].

It is an important consideration that the above experiments were performed in a culture model. Such models are useful for defining individual signaling pathways but the results obtained must be fully evaluated in mouse models. Both polar and apolar modes of presentation are found *in vivo* and luminal morphogenesis can be modeled in a transgenic mouse model [12, 13]. However, the mechanisms that regulate the orientation of epimorphin presentation *in vivo* are unknown. Although experiments with cultured cells showed that release of epimorphin from the cell surface (a prerequisite for apolar presentation to epithelial cells of a

mesenchymally produced protein) could be blocked with inhibitors of matrix metalloproteinases (MMPs) [13] and epimorphin has been shown to induce expression of MMPs in cultured mammary epithelial cells [27], no direct link has yet been established between a particular MMP and epimorphin solubilization.

Showing the way: downstream effects of epimorphin

Epimorphin signaling has been implicated in two mechanisms that are crucial for mammary gland development. First, epimorphin has been shown to stimulate the expression of the transcription factor CCAAT/enhancer binding protein- β (C/EBP β) [13], which is essential for proper mammary morphogenesis and for mammary epithelial cell fate determination [34]. Epimorphin treatment increases the ratio of the shorter isoform of C/EBP β (LIP) relative to the longer isoform (LAP) both in culture and in the epimorphin transgenic mouse, although the mechanism by which this occurs is unclear. However, experimental manipulation of the LIP/LAP ratio in cultured cells can reproduce the epimorphin morphogenic activity in the absence of epimorphin signaling [13].

Second, epimorphin-treated mammary epithelial cells were found to produce increased levels of MMP-2, MMP-3 and MMP-9, and this expression was apparently essential for morphogenic activity because branching of mammary organoids could be completely blocked with inhibitors of MMPs (MMPIs) but not with inhibitors of serine, cysteine or aspartate proteinases [27]. Although addition of MMP-3 was sufficient to induce branching in mammary organelles, it was not sufficient in a mammary cell line that does not make its own basement membrane unless epimorphin was also present. The observation that epimorphin-mediated branching morphogenesis occurs through induced expression of MMPs is relevant for several reasons. First, MMPs have been extensively implicated in side-branching of mammary ducts [21]. Second, MMP-mediated remodeling of the stroma is necessary to make room for and to provide support to the expanding epithelial structures [35]. Third, MMPs have been shown to be involved in the conversion of mammary epithelial cells to a mesenchymal phenotype [36] and transient epithelial-to-mesenchymal transitions are suspected of being necessary for branch initiation in the mammary gland and in other tissues [22,27]. Although these studies make it clear that epimorphin morphogenesis is mediated through C/EBP β and MMPs, the signal transduction pathways connecting epimorphin and these effectors are completely unknown and are an important target of investigation.

Branching out: epimorphin and the morphogenesis of glandular organs

Epimorphin localization has been studied in several different tissues (Table 1) and distinct morphogenic effects have been found in many of these. In addition to its effects on mammary epithelial cells, epimorphin has been found to stimulate luminal morphogenesis of primary gall-bladder [37], pancreas [38] and liver [39,40] epithelial cells. In liver, it also induces functional differentiation [39]. Epimorphin also stimulates branching morphogenesis of lung organoids [28,41] and cultured endothelial cells [42].

It is remarkable to find that a single molecule should be involved in morphogenic processes in so many different tissues (Box 2) but it is becoming clear that this is a common pattern in developmental biology [43]. A growing list of molecules have been found to have morphogenic properties in a wide variety of organs, including HGF [44], TGF β [11], FGFs [25] and MMPs [35]. That epimorphin plays a role in the morphogenesis of so many different glandular tissues (Table 1) suggests that, like these other morphogenic signals, epimorphin might also have been derived from a morphogen signaling network in an evolutionarily primitive organ and that this network was then adapted to additional systems as organisms increased in complexity. This possibility highlights the importance of identifying the other elements of epimorphin signaling pathway, particularly its epithelial cell surface receptor(s).

Concluding remarks

Given that postpubertal mammary gland development is regulated by systemic hormones, one of the most immediate questions is how epimorphin action is coordinated with, or controlled by, hormone signaling. The overlapping roles of the different hormones complicate such investigations; as a starting point, it might be most productive to focus on hormonal signals known to stimulate side-branching, the activity for which epimorphin has been best characterized. In this regard, it is notable that both progesterone and prolactin are involved in ductal side-branching processes [45] and that investigations of C/EBP β knockout mice have revealed that this transcription factor regulates the expression and function of progesterone receptors and prolactin receptors [46]. Given that epimorphin is an upstream regulator of C/EBP β function, this observation offers insight into the potential mechanisms by which epimorphin collaborates to regulate signals for cell proliferation and tissue morphogenesis.

Another major question is how changing the orientation of epimorphin presentation could lead to such different morphogenic consequences. In this regard, insight might be provided by comparison with a similar model system. Growth of Madin–Darby canine kidney (MDCK) cells in 3D collagen leads to cystic structures with central lumina that can be induced to branch in the presence of HGF [22,47]. In this system (as well as in the mammary acini), it has been shown that apoptosis plays an important role in the development of luminal space [48] and it is also likely to do so in the branching/luminal structures produced by epimorphin [13]. However, the morphogenic fate of the MDCK structures has been shown to be controlled through orientation of the mitotic spindle, because division of a cell in parallel with the surface of a spherical cyst produces expansion of luminal diameter, whereas division of a cell perpendicular to the surface of the cyst leads to initiation of a branch point [22,49]. Given that apolar presentation of recombinant epimorphin leads to lumen formation and that polar (basal) presentation of recombinant epimorphin leads to branching formation, it is tempting to propose that epimorphin could provide the morphogenic cue to mitotic spindle orientation, at least in the mammary system (Fig. 5). This possibility also provides insight into the relationship between the activities of epimorphin and syntaxin-2 (Box 1). Syntaxin-2 has been shown to be localized to the apical compartment in some cell types [50,51], implicating it in polarized secretion. It could therefore be that this one molecule provides a link between polarity of secretion and polarity of division in the mammary gland. The definitive test of this possibility will require time-lapse imaging of structures undergoing morphogenesis in which orientation of epimorphin presentation can be controlled. These investigations are currently underway and will probably provide insight into developmental processes in many other organs as well.

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Fig. 1.

Postnatal mammary gland development. Prior to birth, mammary placodes and early anlage form in an organ-autonomous fashion, whereas postnatal development occurs under the control of systemic mammogenic hormones. Ductal elongation during puberty occurs through extensive cell division at terminal end buds (TEBs), specialized structures that can produce both luminal and myoepithelial cell populations. TEBs are lost at maturity and replaced by end buds. The gland becomes functionally differentiated during pregnancy, when extensive proliferation leads to development of lobuloalveoli, followed by conversion of the lobuloalveolar epithelium to a secretory phenotype during lactation. Cessation of nursing leads to milk stasis and consequent glandular involution, in which extensive epithelial apoptosis returns the gland to a state that is similar but not identical to the prepregnant state.

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Fig. 2.

Minimal structural unit of the mammary alveolus. The mammary ductal network is elaborated from a simpler layered structure. The inner layer consists of a heterogeneous population of luminal epithelial cells surrounding a central lumen, whereas the outer layer contains myoepithelial cells surrounded by the basement membrane. This multilayered structure is embedded in the stroma, consisting of a variety of mesenchymal cells in a fibrous extracellular matrix. The precise spatial relationship of epithelial and myoepithelial cells is not constant and changes as a function of hormonal and reproductive status, and some epithelial cells contact the basement membrane.

(a) Bifurcation **(b) Side-branching** *TRENDS in Cell Biology***Fig. 3.**

Alternative methods of branch initiation. (a) Primary branching. Terminal end buds (TEBs) consist of a leading layer of highly proliferative cap cells that differentiate into the luminal epithelial and myoepithelial cells. Branching of TEBs involves bifurcation for separation of the direction of elongation. (b) Side branching. Quiescent ductal structures might initiate side branching, possibly through transient epithelium-to-mesenchyme transition of the luminal epithelial cells, associated with outward migration through the myoepithelial cell layer and degradation of the surrounding basement membrane. These processes can also be distinguished in that elongation of the primary branch and side branches involve different metalloproteinases.

Fig. 4.

Epimorphin directs distinct morphogenic pathways in mammary epithelial cells cultured in three-dimensional collagen. (a) Presentation of recombinant epimorphin to the outer surface of clustered cells (polar, basal presentation) leads to the formation of highly branched structures. (b) Presentation of epimorphin around every cell in the cluster (by addition of exogenous recombinant epimorphin or by secretion from transfected cells; apolar presentation) leads to morphogenesis of cystic structures with large central lumen. We postulate that similar mechanisms might also operate *in vivo* because the expression of epimorphin in different cell types of the mammary gland shifts during the reproductive cycle.

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Fig. 5.

A possible mechanism by which epimorphin presentation could orient mitotic spindles to signal distinct morphogenic pathways. It has been suggested that the orientation of the mitotic spindle during cell division can lead to distinct morphogenic processes [22]. We propose that orientation of epimorphin presentation might in turn control the orientation of the mitotic spindle axis. If this is so then polar presentation of epimorphin (a) could lead to division perpendicular to the plane of the epithelial layer, to initiate branching morphogenesis, whereas apolar presentation of epimorphin (b) could lead to parallel division, to increase the luminal diameter.

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Fig. 1.

(a) Syntaxin-mediated vesicle fusion. In the nonreactive state (left), syntaxin-1a (syn1a; yellow, orange and red) forms a four-helix bundle in association with nSec1 (gray) (structure adapted from Ref. [81]). Vesicle fusion (right) is triggered by association of the syn1a SNARE domain (red) with SNAP25 (blue) and vesicular synaptobrevin (green) (structures adapted from Refs [79,81]); formation of this four-helix bundle is not believed to involve the epimorphin homologous domain (yellow). (b) Functional domains of syntaxins and epimorphin. Structural studies of syntaxins have revealed a protein in which three β -helices (yellow) are connected by a linker (orange) to the SNARE helix and a C-terminal transmembrane domain (red). Deletion analyses of syntaxins have shown that the SNARE domain is necessary and sufficient to mediate vesicle fusion. Deletion analyses of epimorphin, however, reveal that the N-terminal helices are essential for morphogenic activity, whereas the SNARE domain is dispensable [12].

Table 1

Epimorphin expression and activity

Tissue-specific expression	Refs
Tooth epithelium	[52]
Hair follicles	[53,54]
Venous endothelium	[42]
Pancreatic β cells	[55]
Liver epithelium	[39,40]
Intestinal epithelium	[56,57]
Skin keratinocyte	[58]
Lung epithelium	[59–61]
Kidney epithelium	[62]
Mammary epithelium	[12,32]
Defined role in morphogenesis	[28]
Hair follicle development	[28,41]
Lung epithelium branching	[39]
Liver sinusoid formation	[42]
Endothelial tubulogenesis	[40]
Liver regeneration	[63]
Skin keratinocyte reciprocal interaction	[37]
Gallbladder epithelium lumen formation	[38]
Pancreatic epithelium lumen formation	[12,27,32]
Mammary epithelium branching and lumen formation	[64]
Intestinal-crypt villus axis formation	[52]

Table I

Molecules secreted by non-classical pathways that possess distinct intracellular and extracellular function

Intracellular/extracellular name	Intracellular function	Extracellular function
Phosphohexose isomerase/ autocrine motility factor	Glycolysis, gluconeogenesis [99,100]	Tumor autocrine motility factor, neurotrophic factor [99,100]
RHAMM/CD168	Cytoskeletal component [101]	Hyaluronic acid receptor [102]
Galectin-1	Association with Ras; mRNA splicing [103]	Immunosuppressive cytokine [104]
HMGB1/amphoterin	Architectural DNA-binding protein [105]	Pro-inflammatory cytokine [106,107]
Tissue transglutaminase	G-Protein [108]	Protein cross-linker [109]; cell–extracellular matrix interaction [110]
Thioredoxin/ADF	Oxidoreductase [111]	Immunomodulatory cytokine [111]
Syntaxin-2/epimorphin	Membrane fusion	Morphogenesis