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Mammary gland development—It's not just about estrogen[1]

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ABSTRACT

The mammary gland (MG) is one of a few organs that undergoes most of its growth after birth. Much of this development occurs concurrently with specific reproductive states, such that the ultimate goal of milk synthesis and secretion is coordinated with the nutritional requirements of the neonate. Central to the reproductive–MG axis is its endocrine regulation, and pivotal to this regulation is the ovarian secretion of estrogen (E). Indeed, it is widely accepted that estrogens are essential for growth of the MG to occur, both for ductal elongation during puberty and for alveolar development during gestation. As the factors regulating MG development continually come to light from the fields of developmental biology, lactation physiology, and breast cancer research, a growing body of evidence serves as a reminder that the MG are not as exclusively dependent on estrogens as might have been thought. The objective of this review is to summarize the state of information regarding our understanding of how estrogen (E) has been implicated as the key regulator of MG development, and to highlight some of the alternative E-independent mechanisms that have been discovered. In particular, we review our findings that dietary *trans*-10,*cis*-12 conjugated linoleic acid promotes ductal elongation and that the combination of progesterone (P) and prolactin (PRL) can stimulate branching morphogenesis in the absence of E. Ultimately, these examples stand as a healthy challenge to the question of just how important estrogens are for MG development. Answers to this question, in turn, increase our understanding of MG development across all mammals and the ways in which it can affect milk production.

Key words: prolactin, progesterone, mammary epithelial, conjugated linoleic acid

INTRODUCTION

The mammary glands (**MG**) are unique among organs with respect to the large amount of postnatal development they undergo. The extent of epithelial proliferation within the gland is massive and often occurs during a very short interval; this topic has been reviewed extensively elsewhere (Hovey et al., 2002; Yart et al., 2014). These periods of allometric growth coincide with critical periods of reproductive development. The first is the beginning of allometric growth around the onset of puberty. Depending on species, growth in this period accomplishes expansion of the parenchymal ductal network via elongation and arborization of the ductal system. The second phase initiates specifically during pregnancy when the parenchyma expands allometrically in association with expansion of the alveolar population in anticipation of lactogenesis.

THE CASE FOR ESTROGENS AS PRIMARY REGULATORS OF MG DEVELOPMENT

There are many clear demonstrations that the ovaries are essential for the normal MG to develop, not least the fact that ovariectomy (**OVX**) of females from a variety of species, including rodents (Hovey et al., 2002), ruminants (Berry et al., 2003; Yart et al., 2014), and nonhuman primates (Cline et al., 1996), halts MG growth. An interesting exception appears to be sheep, which continue to undergo MG growth during the prepubertal period following OVX (Ellis et al., 1998).

Estrogens (**E**) are key contributors to the 2 aforementioned phases of allometric MG development. During elongation of the ductal network around the onset of puberty, activation of the hypothalamic–pituitary–gonadal axis leads to increased epithelial proliferation at the termini of ducts, either as terminal end bud (**TEB**) structures characteristically present at the leading edge of the mammary ducts in rodents, pigs, and humans (Hovey et al., 2002; Rowson et al., 2012) or as a peripheral zone of mitosis within the more complex terminal ductal lobular unit structures found within the MG of female ruminants such as heifers and ewe lambs (Ellis and Capuco, 2002). The ability of E to promote TEB formation in rodents is particularly pronounced: de-

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pending on the dose and route of exposure, the mitotic rate in epithelial cells following E exposure increases from <1 to >50% in a matter of days (Daniel et al., 1987). In heifers, the effect of OVX or exogenous E on mammary epithelial cell (**MEC**) proliferation is much less pronounced; OVX reduced the rate of mitosis from ~3 to ~1% (Meyer et al., 2006a), whereas exogenous E only increased the rate of mitosis from ~8 to ~14% (Capuco et al., 2002) or from ~3 to ~8% (Meyer et al., 2006a). During gestation, there is an equal requirement for estrogens to realize full lobulo-alveolar development, where hormone replacement experiments in rats demonstrated that the combination of E, progesterone (**P**), prolactin (**PRL**), and growth hormone (**GH**) was required for full development to occur (Lyons et al., 1958).

THE CASE FOR ESTROGEN-INDEPENDENT PATHWAYS DURING MG DEVELOPMENT

As summarized above, estrogens have undeniable effects on the MG epithelium. However, examples also exist that raise the question of whether MEC proliferation and development of the MG can occur independently of estrogens or at least with only partial dependence on them. The following review seeks to highlight these examples.

The In Vitro Paradox

Growth of the MG ultimately reflects an increase in the number of MEC within the parenchyma. In turn, the ability to grow and culture these cells provides an important tool for studying the regulation for normal MEC growth. However, one discrepant issue has been the longstanding inability to replicate several aspects of E-induced mammary growth in vitro.

An analysis of E receptor (**ER**) expression in vivo indicates that most ER within the MG are expressed by MEC, primarily in a luminal population (Anderson et al., 1998) and are also expressed by cells within the supporting adipose stroma (Hovey et al., 1999). The presence of epithelial ER raises the question of their function, where one might surmise that treatment of isolated MEC with E would promote their proliferation. However, this expectation is not the case for most studies of normal MEC in vitro. When normal MEC isolated from different species, including mice and humans, were treated with E, they failed to demonstrate a robust proliferative response over a range of E concentrations (Haslam and Levely, 1985; Richards et al., 1988; Xie and Haslam, 1997). One consistent observation during these studies is that normal MEC frequently lose most of their ER expression upon their isolation and during culture (Xie and Haslam, 1997), possibly explaining the loss of E-responsiveness in vitro. An alternative hypothesis is that ER within the surrounding stromal environment, or these stromal cells in some other capacity, are required to mediate a proliferative effect of E, where exogenous E promotes a parallel proliferative burst in stromal cells surrounding the epithelium of mice (Woodward et al., 1998). Interestingly, in heifers, a round of E-induced stromal proliferation occurs after the phase of epithelial proliferation (Woodward et al., 1993). Several lines of evidence suggest that stromal cells (Haslam and Levely, 1985) or extracellular matrix proteins (Novaro et al., 2003) are required to confer a proliferative response to E by MEC in vitro. In many other ways, however, the magnitude of the E-induced proliferative response in the mouse MG in vivo is yet to be recapitulated in vitro. As a case in point, even when entire intact MG from mice are cultured in vitro using a whole-organ culture system, they fail to undergo ductal elongation or formation of TEB in response to supplemental E, in the presence or absence of GH or IGF-1, despite having a full complement of epithelial and stromal cell types (Vonderhaar, 1984). The suggestion that the effects of E on the MG may not be direct is further emphasized by responses to hormone priming of donor mice before organ culture; priming nulliparous mice with either E or P fails to facilitate hormone-induced responsiveness, which can only be achieved by priming donor animals with both E and P. Thus, the paradox exists that E, either alone or with its mediators IGF-1 and GH, cannot fully recapitulate the growth-promoting effects in vitro that it promotes in vivo.

A Paradox for the Role of E During Ductal Elongation In Vivo

Despite a clear role for E during allometric growth of the MG, a series of classic experiments highlighted that in vivo, E alone is ultimately unable to promote growth of the MG. Specifically, whereas abolition of MG development by OVX could be rescued by exogenous E, this response could not be achieved in females that were hypophysectomized (Lyons et al., 1958; Nandi, 1958). Subsequent experiments confirmed the combined requirement for the ovarian hormone E and pituitary GH during ductal development in rodents and that these hormones synergistically induce local synthesis of IGF-1 in the stromal microenvironment (Kleinberg and Ruan, 2008). In turn, exogenous IGF-1 can stimulate the proliferation of MEC in vivo to recapitulate the formation of TEB (Kleinberg and Ruan, 2008). The important conclusion from these experiments was that IGF-1 is the primary effector of mammary ductal growth during