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Hepatocyte growth factor-like protein is a positive regulator of early mammary gland ductal morphogenesis

Devikala Gurusamy [a,1], *Sasha J. Ruiz-Torres* [a,1], *Abby L. Johnson* [b,1], *Dana A. Smith* [a], *Susan E. Waltz* [a,c,*]

[a] *Department of Cancer Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0521, USA*
[b] *Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0521, USA*
[c] *Research Service, Cincinnati Veterans Hospital Medical Center, Cincinnati, OH 45267-0521, USA*

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ABSTRACT

The Ron receptor tyrosine kinase regulates multiple cellular processes and is important during mammary gland development and tumor progression. Hepatocyte growth factor-like protein [HGFL] is the only known ligand for the Ron receptor and recent studies have identified major roles for HGFL during breast cancer metastasis. Understanding the functional importance HGFL during mammary gland development will provide significant insights onto its contribution during tumor development and metastasis. In this study, we assessed the role of HGFL during postnatal mammary gland development using mice that were either proficient [HGFL +/+] or deficient [HGFL−/−] for HGFL. Postnatal ductal morphology and stromal cell associations were analyzed at multiple time points through puberty until adulthood. HGFL deficiency resulted in several mammary gland developmental defects including smaller terminal end buds [TEBs], significantly fewer TEBs, and delayed ductal outgrowth during early puberty. Additionally, HGFL deficient animals exhibited significantly altered TEB epithelial cell turnover with decreased proliferation and increased apoptosis coupled with decreased TEB diameter. Macrophage recruitment to the TEBs was also significantly decreased in the HGFL−/− mice compared to controls. Moreover, the levels of STAT3 mRNA as well as the phosphorylation status of this protein were lower in the HGFL−/− mammary glands compared to controls. Taken together, our data provide the first evidence for HGFL as a positive regulator of mammary gland ductal morphogenesis by controlling overall epithelial cell turnover, macrophage recruitment, and STAT3 activation in the developing mammary gland. With a function in early mammary gland development, HGFL represents a potential target for the development of novel breast cancer therapies.

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* Corresponding author at: University of Cincinnati College of Medicine, Department of Cancer Biology, 3125 Eden Avenue, Cincinnati, OH 45267-0521, USA. Tel.: +1 513 558 8675; fax: +1 513 558 4428.
E-mail address: susan.waltz@uc.edu (S.E. Waltz).
[1] These authors contributed equally to this work.
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1. Introduction

The mammary gland is a complex structure composed of epithelial and stromal cells that include fibroblasts, adipocytes, endothelial cells, nerve cells, and migratory leukocytes [macrophages and eosinophils] (Gouon-Evans et al., 2002). The development of a mammary gland is a highly regulated and intricate process that occurs throughout the life of an animal, beginning in the embryo and continuing postnatally during puberty, pregnancy, lactation, and involution (Richert et al., 2000). During embryonic development, a rudimentary mammary gland ductal structure invades the mesenchymal tissue and remains dormant until approximately 21 days of age, where the onset of ovarian hormone secretions stimulate ductal growth (Richert et al., 2000). Terminal end bud [TEB] structures are found exclusively in the developing mammary gland and are the main driving force for mammary gland development. During puberty, TEB formation and side branching drive mammary gland epithelial cell invasion into the mammary fat pad (Sternlicht, 2006). Additional primary ducts are formed through bifurcation of existing TEBs. Trailing ducts sprout secondary branches, while short tertiary branches form off of the developed secondary branches (McNally and Martin, 2011). This extension of ductal branching into the surrounding fat pad continues until the entire fat pad is filled.

Pubertal mammary gland morphogenesis integrates a balance of epithelial cell proliferation, differentiation, and apoptosis (McNally and Martin, 2011). In addition, several studies have identified the interactions between mammary epithelial cells, mesenchymal cells, and leukocytes to be crucial for the proper postnatal development of the mammary ductal tree (Gouon-Evans et al., 2000, 2002; Sternlicht, 2006; Wiseman and Werb, 2002). Interestingly, studies have shown that processes important in mammary gland development are often deregulated during breast cancer tumorigenesis (Lanigan et al., 2007; Micalizzi et al., 2010). Thus, understanding the complex signaling network as well as the interactions between the different cell types during mammary gland development will be vital for elucidating the mechanisms underlying breast cancer progression and metastasis.

Mammary gland development is dependent on many growth factors that target receptor tyrosine kinases, including epidermal growth factor [EGF], insulin-like growth factor [IGF], and hepatocyte growth factor [HGF] (Garner et al., 2011). EGFR, IGFR, and HGFR [also known as c-Met], the tyrosine kinase receptors associated with these growth factors, have also been found to be associated with poor prognosis in breast cancer (Chrysogelos and Dickson, 1994; Lengyel et al., 2005; Resnik et al., 1998). In studies with EGFR impaired kinase activity, a decrease in branching and ductal extension and hence overall mammary gland development was observed compared to wild type controls (Sebastian et al., 1998). Studies using mice that lacked the IGFR ligand, IGF, showed that mammary gland development was not possible without IGF, suggesting its central role in this process (Ruan and Kleinberg, 1999). Using a conditional deletion of c-Met receptor that inhibited HGF signaling, it was shown that loss of HGF signaling leads to a 35% reduction in overall branching morphogenesis (Garner et al., 2011). Hepatocyte growth factor-like protein [HGFL] shares 45% amino acid homology to HGF (Wagh et al., 2008). Because of the similarities between HGF and HGFL and the established importance of growth factors and their associated receptor tyrosine kinases during mammary gland development, we chose to study the effects of HGFL in this context.

The Ron receptor tyrosine kinase, a member of the c-Met family of receptor tyrosine kinases, is overexpressed in about 50% of primary breast cancers (Wagh et al., 2008). Previously, our laboratory has shown the Ron receptor to be important during both mammary gland development (Meyer et al., 2009) as well as during breast cancer tumorigenesis (McClaine et al., 2010; Peace et al., 2005; Zinser et al., 2006). HGFL, also known as macrophage stimulating protein [MSP], is the only known ligand for Ron (Bezerra et al., 1993; Wang et al., 1994a) and was initially identified as a chemotactic protein capable of inducing macrophage shape change, chemotactic migration, and phagocytosis (Leonard and Skeel, 1976). In a recent study, HGFL was also shown to function as a macrophage chemoattractant in a rat kidney inflammatory model (Rampino et al., 2007).

HGFL is predominantly produced by the hepatocytes and is secreted into the bloodstream as an inactive single chain polypeptide precursor, pro-HGFL. Pro-HGFL then works in an endocrine fashion, when locally cleaved by proteases of the coagulation cascade (Leonard and Danilkovitch, 2000; Wang et al., 1994b) or by matriptase expressed on macrophages (Bhatt et al., 2007), to form an active heterodimer. HGFL-dependent Ron activation results in receptor dimerization and trans-autophosphorylation of key tyrosine residues, leading to activation of downstream signaling targets such as STAT3, Akt, MAPK, and β-catenin, which are shown to be important in both mammary gland development and breast cancer tumorigenesis (Wagh et al., 2008). Interestingly, Ron activation has been shown to increase STAT3 phosphorylation (Danilkovitch-Miagkova, 2003; Gurusamy et al., 2013) and mammary epithelial-specific STAT3 activation has been shown to regulate the number of macrophages recruited to the developing mammary gland (Hughes et al., 2012). Furthermore, STAT3 in macrophages has been shown to regulate the production of inflammatory cytokines and ultimately the tissue microenvironment (Akira, 2000). Interestingly, the coordinated expression of Ron, HGFL, and the protease matriptase has been shown to be a strong independent indicator of both metastasis and poor prognosis in breast cancer patients (Welm et al., 2007). In addition, HGFL expression by tumor cells has been shown to increase the spectrum of metastasis using an orthotopic mouse model of breast cancer (Welm et al., 2007).

Although the Ron receptor was previously shown to be a critical negative regulator of mammary gland ductal morphogenesis (Meyer et al., 2009), the importance of HGFL during mammary gland development was not assessed. In this study, we hypothesized that loss of HGFL will augment mammary gland ductal morphogenesis, similar to the phenotype observed with Ron loss. Our data reported here documents

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