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Biological Role of the HGF/MET Ligand/Receptor Couple in Bovine Mammary Epithelial Cells

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Abbreviations: FBS, foetal bovine serum; HGF, Hepatocyte Growth Factor

INTRODUCTION

The mammary gland is an organ in which numerous remodelling events follow each other during the development and the reproductive cycle of the animal. After puberty and during pregnancy morphogenesis and proliferation transform the primordial epithelium to an extended network composed of hollow tubules and secreting acini.

The development of the mammary gland requires several locally-derived growth factors such Hepatocyte Growth Factor (HGF). Hepatocyte Growth Factor (HGF) is a cytokine originally described as a mitogenic factor for hepatocytes during liver regeneration. HGF is secreted by mesenchymal/stromal cells and acts as a paracrine factor on adjacent epithelial cells that express the c-met tyrosine kinase receptor (Sonnenberg *et al*., 1993). Binding of HGF to its receptor induces multiple biological responses including proliferation, motility, invasion of the extracellular matrix, resistance to apoptosis and activation of angiogenesis (Bussolino *et al*., 1992; Brinkmann *et al*., 1995; Bardelli *et al*., 1996; Medico *et al*., 1996). HGF and c-met are expressed and temporally regulated during mammary development and differentiation; some mammary epithelial cell lines grown in a three dimensional collagen matrix with HGF generate tubules (Soriano *et al*., 1995) with a morphology resembling mammary ducts.

Recently, bovine HGF and c-met have been analyzed for their expression in the mammary gland, but no data regarding their biological roles are yet available. We have therefore investigated whether the bovine mammary epithelial cell line BME-UV1 was responsive to HGF and what the biological effects induced by this cytokine were.

MATERIALS AND METHODS

Bovine mammary epithelial cell line BME-UV1 (a kind gift of Prof. I. Politis, Department of Animal and Food Sciences, University of Vermont, USA) was maintained in

DMEM supplemented with 10 % FBS (Euroclone) and 10 μg/ml insulin (Sigma) and were incubated at 37°C in a 5 % CO_2-water-saturated atmosphere. Recombinant HGF, EGF and IGF-I (Immunotools) were used at a concentration of 10 ng/ml, 10 ng/ml and 100 ng/ml, respectively. Real Time PCR assays were performed as described in (Hecht *et al*., 2005). Proliferation assays were performed plating 4×10^3 BME-UV1 in 96-well plates; following 24 hours incubation the medium was replaced with DMEM 1 % FBS with the different growth factors and 48 hours later proliferation was evaluated with an MTT kit (Roche) following the manufacturer's instructions. Western blot analysis was performed as described in (Maritano *et al*., 2000). The invasion assay (6.5 mm diameter, 8 μm pore size transwells, Costar) as described in (Taulli *et al*., 2005). For the scatter assay 3×10^3 BME-UV1 were plated in 96-well plates and after 24 hours the medium was replaced with DMEM 1 % FCS medium with the indicated growth factors; after 16 hours cells were fixed with 11 % glutaraldeyde, stained with cresyl violet (Sigma) and photographed on an inverted microscope. The tubulogenesis assays were performed as described in (Montesano *et al*., 1991).

RESULTS

As the first step we verified that bovine mammary epithelial cell line BME-UV1 express the tyrosine kinase receptor c-met mRNA by Real Time PCR using bovine specific oligonucleotides that anneal on different exons. The expression level of BME-UV Met mRNA was higher than that of two other mouse mammary epithelial cell lines (TAC-2 and HC-11).

Proliferation of BME-UV1 cells following HGF induction was significantly higher than that obtained with all other cytokines tested (HGF 191 %, EGF 123 %, IGF-I 143 %, Dexamethasone 148 % *vs* control 100 %). HGF was the only tested cytokine able to induce cellular motility and dispersion (the phenomenon defined as "scatter"). Migration and degradation of the extracellular matrix (transwell assay) was only significantly induced by HGF. This feature, although typical of invading cancerous cells, is also of fundamental importance during the development of the mammary gland, an event in which mammary epithelial cells invade the surrounding stroma and generate new tubules.

After HGF addition BME-UV1 responded biochemically activating the transductional pathways of MAPK (a proliferative pathway) and Akt (an anti-apoptotic pathway). BME-UV1 cells grown in a tridimensional collagen matrix were able, although to a limited extent, to develop tubules, when HGF was added to the culture medium.

DISCUSSION

Tubulogenesis and ramification represent a complex morphogenetic process, particularly active in the developing mammary gland, that requires efficient coordination between cellular proliferation, polarity and movement. The study of these events and their driving factors is part of an interesting and current field of research. The bovine tyrosine kinase

receptor c-met and its ligand HGF have been recently cloned and their expression in the developing mammary gland has been analyzed (Yamaji *et al*., 2006) but the biological properties of HGF on mammary epithelial cell lines have not yet been illustrated. The effects described in this work show how HGF and its receptor c-met may have a significant role on the physiology of the mammary gland. The data reported demonstrate how c-met activation in bovine mammary epithelial cells induces multiple biological responses such as proliferation, motility and morphogenesis, all fundamental in the tubulogenesis event. These responses are fundamental in both the physiological and pathological events, particularly tumors that can develop in the mammary gland.

Other analyses are still under way to understand the possible interactions between HGF and other growth and differentiation factors expressed in the mammary gland, in particular during pregnancy, the moment of maximal development of this organ.

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