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Huntingtin Regulates Mammary Stem Cell Division and Differentiation

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SUMMARY

Little is known about the mechanisms of mitotic spindle orientation during mammary gland morphogenesis. Here, we report the presence of huntingtin, the protein mutated in Huntington's disease, in mouse mammary basal and luminal cells throughout mammogenesis. Keratin 5-driven depletion of huntingtin results in a decreased pool and specification of basal and luminal progenitors, and altered mammary morphogenesis. Analysis of mitosis in huntingtin-depleted basal progenitors reveals mitotic spindle misorientation. In mammary cell culture, huntingtin regulates spindle orientation in a dynein-dependent manner. Huntingtin is targeted to spindle poles through its interaction with dynein and promotes the accumulation of NUMA and LGN. Huntingtin is also essential for the cortical localization of dynein, dynactin, NUMA, and LGN by regulating their kinesin 1-dependent trafficking along astral microtubules. We thus suggest that huntingtin is a component of the pathway regulating the orientation of mammary stem cell division, with potential implications for their self-renewal and differentiation properties.

INTRODUCTION

There are three distinct and differentially regulated stages in mammary gland development (embryonic, pubertal, and pregnancy/lactation), and the most substantial remodeling is postnatal (Gjorevski and Nelson, 2011). The mammary epithelium is organized into two cell layers: the luminal and basal myoepithelial layers. During pregnancy, the mammary gland completes its morphogenesis with the formation of alveolar buds where milk production is turned on at the end of pregnancy and during lactation (Silberstein, 2001). This developmental process is controlled by steroid hormones (Beleut et al., 2010). During lactation, luminal cells (LCs) produce and secrete milk, whereas basal myoepithelial cells (BCs) contract to release the milk from the nipple (Moumen et al., 2011).

Several lines of evidence indicate the existence of mammary stem cells (MaSCs) in mouse mammary tissue. These cells display the regenerative properties required for the substantial developmental changes in the adult mammary gland (Visvader and Lindeman, 2011). MaSCs have been isolated from adult mouse mammary tissue using the surface markers CD24 and β1 or α6-integrin chains (Shackleton et al., 2006). These populations are negative for steroid hormone receptors and consist of cells that express basal cell markers (Asselin-Labat et al., 2010). However, these populations appear to be composed of various subpopulations, ranging from multipotent stem cells to terminally differentiated luminal epithelial and myoepithelial cells (Visvader and Lindeman, 2011). Furthermore, the LC compartment itself is heterogeneous because progenitors of varying states of luminal differentiation and with diverse proliferative capacities can be identified (Shehata et al., 2012).

The importance of asymmetric cell divisions for stem cells/progenitors has been established in several tissues (Morin and Bellaïche, 2011; Shitamukai and Matsuzaki, 2012). In the mouse mammary gland, the reproductive cycle may alter the MaSC population by regulating the balance between symmetric and asymmetric divisions (Asselin-Labat et al., 2010; Joshi et al., 2010). Experimental perturbation of this balance results in abnormal epithelial morphogenesis and favors tumor growth (Cicalese et al., 2009; Taddei et al., 2008). Thus, MaSC divisions are important regulators of physiological and pathological stem cell biology. However, the precise molecular mechanisms underlying the division modes in mitotic MaSCs are still not understood.

The mitotic spindle is a key component of cell division. The position and orientation of the mitotic spindle are orchestrated by forces generated in the cell cortex (Grill and Hyman, 2005), where astral microtubules emanating from the mitotic spindle pole are tethered to the plasma membrane (Siller and Doe, 2009). Spindle orientation is determined by an evolutionarily conserved pathway, including cytoplasmic dynein, dynactin, the nuclear mitotic apparatus (NUMA) protein, and the G protein regulator leucine-glycine-asparagine repeat (LGN) protein (the

vertebrate homolog of *Caenorhabditis elegans* G protein-coupled receptor (GPR-1)/GPR-2 and *Drosophila* protein-protein interaction networks [PINS]) (Morin and Bellaïche, 2011). During cell division, LGN is recruited to the cell cortex through glycosyl phosphatidylinositol-linked $G_{\alpha i}/G_{\alpha o}$, which binds LGN carboxy-terminal GoLoco motifs (Zheng et al., 2010). Polarity cues restrict LGN localization to specific subcortical domains, where LGN recruits NUMA (Peyre et al., 2011). NUMA in turn interacts with microtubules and with the cytoplasmic dynein/dynactin complex. The precise localization of these interactions at the cell cortex ensures the positioning of the mitotic spindle through cortical capture of astral microtubules. Although these mechanisms have been well described in the skin and neuroepithelium, their involvement in the division of MaSCs is not known.

We previously showed that huntingtin (HTT), the protein mutated in Huntington's disease (HD), is required in murine neuronal progenitors for appropriate spindle orientation and for cell fate determination (Godin et al., 2010). Yet, the mechanisms underlying HTT function during spindle orientation remain unclear. HTT expression is not restricted to the brain: mutant HTT is detected in healthy mammary tissue and mammary tumors where it regulates tumor progression (Moreira Sousa et al., 2013). Thus, HTT may contribute to spindle orientation and cell fate choices outside the nervous system. Here, we investigated the function of HTT in mitosis of MaSCs during mouse mammary epithelium morphogenesis.

RESULTS

In Vivo Depletion of HTT from the Basal Compartment Leads to a Decreased Epithelial Content and Alters Self-Renewal of the Basal and Luminal Progenitors

We analyzed the expression pattern of wild-type HTT in mammary glands from virgin mice by immunohistochemistry. HTT immunoreactivity was observed in the basal and luminal compartments and increased as differentiation progressed (Figure 1A). We isolated basal and luminal epithelial cells from wild-type mice using flow cytometry (Figure S1A available online; Table S1). Evaluation of basal (*Krt14*) and luminal (*Krt18*) marker expression by quantitative real-time RT-PCR confirmed that BCs and LCs were found in the CD24-low/α6-high and CD24-high/α6-low fractions, respectively (Figure S1B). HTT was detected in BCs and LCs, but the signal was strongest in the luminal fraction (Figure 1B).

To test whether HTT regulates BC division and differentiation, we deleted HTT from the basal cell layer of the mammary epithelium by crossing $Htt^{flox/flox}$ mice harboring floxed *Htt* alleles (Dragatsis et al., 2000) with transgenic mice expressing *Cre* recombinase under the control of the keratin 5 (K5) promoter (Ramirez et al., 2004). *Cre* expression was mostly confined to the basal cell population (Figure S1C). We analyzed the distribution of HTT-deficient cells in mutant mammary epithelium by crossing K5*Cre*;$Htt^{flox/flox}$ mice with the *Rosa26-LacZ* reporter mouse strain (*R26*). At age 12 weeks, virtually all BCs were *LacZ* positive, whereas only 32% of LCs expressed *LacZ* (Figure 1C). The *LacZ*-negative LC population in the mutant epithelium may originate during early stages of gland development, from *LacZ*-negative BCs and from *LacZ*-negative cells committed to luminal differentiation that switched off the K5 promoter and escaped HTT deletion. Indeed, in embryonic day 18 (E18) K5*Cre*;$Htt^{flox/flox}$;*R26* embryos, a majority of cells in the central part of the developing mammary ducts did not express the *Cre* recombinase (Figure S1D). These cells expressed keratin 8 (K8) and were negative for K5, thus displaying luminal features (Figure S1E). Alternatively, the *LacZ*-negative LC population in the mutant epithelium may originate during adulthood from bipotent myoepithelial and luminal stem cells (Rios et al., 2014). *Htt* transcript levels were 79% lower in mutant than wild-type BCs (Figure 1D). In LCs from mutant mammary epithelium, *Htt* expression levels were 39% lower than the control value (Figure 1D).

Fewer epithelial cells could be isolated from the mutant than control mammary glands (Figures 1E and 1F). Also, the ratio between basal and LC populations was altered in mutant mammary epithelium (Figure 1G). We then performed a functional evaluation of progenitor cell content in control and mutant BCs using colony-formation assay. Mutant BCs formed significantly less colonies than control cells (0.84% ± 0.23% versus 3.32% ± 0.3%, Figure 1H). Similarly, the HTT-depleted LCs failed to form clonal colonies as compared to the control LC population (1.55% ± 0.72% versus 18.6% ± 2.3%, Figure 1I). Thus, K5-driven depletion of HTT leads to gland hypoplasia and affects colony-forming stem/progenitor populations in basal and luminal compartments.

HTT Is Required for Basal and LC Specification

We then analyzed the transcripts of genes associated with proliferation and myoepithelial and luminal lineages (Figure 2A; Table S1). The lower than control levels of the cell proliferation marker *Ki67* in BCs and LCs from mutant glands were consistent with the decrease in the overall population of epithelial cells. In the basal compartment in mutants, whereas *Krt18* was upregulated, *Krt14* and *Trp63* were differentially regulated with *Krt14* being increased and *Trp63* decreased (Figure 2A). In LCs from the mutant glands, both *Krt14* and *Trp63* were increased, whereas *Krt18* was decreased. Also, the expression levels of the