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TAF4b promotes mouse primordial follicle assembly and oocyte survival

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ABSTRACT

Primary ovarian insufficiency (POI) affects 1% of women under the age of 40 and is associated with premature ovarian follicle depletion. TAF4b deficiency in adult female mouse models results in hallmarks of POI including stereotyped gonadotropin alterations indicative of early menopause, poor oocyte quality, and infertility. However, the precise developmental mechanisms underlying these adult deficits remain unknown. Here we show that TAF4b is required for the initial establishment of the primordial follicle reserve at birth. Ovaries derived from TAF4b-deficient mice at birth exhibit delayed germ cell cyst breakdown and a significant increase in Activated Caspase 3 staining compared to control ovaries. Culturing neonatal TAF4b-deficient ovaries with the pan-caspase inhibitor ZVAD-FMK suppresses the excessive loss of these oocytes around the time of birth. These data reveal a novel TAF4b function in orchestrating the correct timing of germ cell cyst breakdown and establishment of the primordial follicle reserve during a critical window of development.

Introduction

At birth, the mammalian ovary contains a finite number of oocytes, most of which will die during the process of follicle development. In contrast, a small number of high quality oocytes are destined for ovulation during adulthood (Jones and Pepling, 2013). Proper regulation of the initial primordial follicle pool is critical for the ovary to maintain sufficient numbers of oocytes for long-term ovulation and fertility. Disruption of the normal balance between oocyte survival and death usually leads to a reduction of the ovarian reserve and impaired fertility (reviewed in McLaughlin and McIver (2009)). While many effectors of oocyte death in the ovary are known, the upstream transcriptional regulators that ensure for proper primordial follicle number and survival at birth, and their associated mechanisms, remain largely unknown. Towards this goal, we are studying the role the transcriptional co-activator TAF4b during oogenesis and folliculogenesis using a TAF4b-deficient mouse model (Freiman et al., 2001).

TAF4b is a gonadal-enriched subunit of the TFIID complex that is critical for female fertility in the mouse. TFIID is a multi-protein general transcription factor composed of the TATA-box binding protein (TBP) and 14 TBP-associated factors (TAFs) (Freiman et al., 2001). TAF4b-deficient female mice are infertile and suffer from hallmarks of premature ovarian aging including persistent estrous, elevated serum follicle stimulating hormone (FSH) and reduced primordial follicle numbers (Voronina et al., 2007; Lovasco et al., 2010). In addition, the human gene encoding TAF4b has been linked to primary ovarian insufficiency (POI) in women (Knauff et al., 2009) as well as human oocyte quality (Di Pietro et al., 2008). Thus, the study of TAF4b in the mouse is highly relevant to the potential role of TAF4b in the proper regulation of human ovarian follicle assembly and survival.

The fidelity of each step of mammalian oogenesis and folliculogenesis is essential for proper oocyte development, ovulation and the developmental potential of the future embryo. The embryonic timeline of murine primordial follicle assembly is well-documented, beginning with germ cell clusters formed through incomplete cytokinesis during mid-embryogenesis. These clusters constitute “cysts” of oogonia, many of which remain associated by intercellular bridges (Pepling and Spradling, 1998; Pepling, 2006; Pepling, 2012a,b) or cyst aggregation (Mork et al., 2012). At embryonic day (E) 13.5, mitosis ceases, and oogonia enter into meiosis I, becoming “oocytes” which usually arrest in the diplotene stage of Prophase I around the time of birth. Concurrently, cysts of germ cells begin undergoing “breakdown”, in which most of the oocytes are lost through programmed cell death (reviewed in Pepling (2012a,b)). In contrast, the remaining

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oocytes become surrounded by a single layer of somatic pre-granulosa cells, forming "primordial follicles". While seemingly simple, this process of primordial follicle assembly requires the intricate orchestration of somatic cell invasion into oogonial cysts, tightly controlled changes in hormone levels, and a unique Caspase 2-dependent apoptotic cascade (Bergeron et al., 1998; Morita et al., 2001; Takai et al., 2007; Lobascio et al., 2007; Tingen et al., 2009). Cyst breakdown is hypothesized to result from inherent ovarian quality control mechanisms, pre-clearing defective germ cells from the future ovarian reserve. In addition, germ cells that do not receive adequate somatic investment for survival are removed by apoptosis (Tingen et al., 2009). However, the molecular and cellular mechanisms required for proper primordial follicle assembly and survival remain largely unknown.

One known regulator of female germ cell cyst breakdown and proper primordial follicle assembly is estrogen (Lei et al., 2010). Estrogen is a steroid hormone that plays important roles in normal ovarian development by promoting granulosa proliferation and differentiation. 17β-estradiol (β-estradiol) is the most bioactive form of endogenous estrogen and is produced by granulosa cells of the ovary. By regulating follicle growth and maturation, β-estradiol modulates the action of FSH and facilitates further β-estradiol production (Drummond and Fuller, 2012). Furthermore, β-estradiol has been shown to promote germ cell survival during the late embryonic and early neonatal period, suggesting that separation from maternal estrogen around the time of birth may facilitate cyst breakdown and associated apoptosis (Chen et al., 2009; Karavan and Pepling, 2012). In addition, aberrant activation of estrogen signaling by genistein (Jefferson et al., 2006; Chen et al., 2007) or diethylstilbestrol (Muñoz-de-Toro et al., 2011; Karavan and Pepling, 2012) perturbs normal cyst breakdown, leading to multiple oocyte follicles. Moreover, estrogen receptors have been shown to be expressed in the somatic cells of the rodent ovary (Fitzpatrick et al., 1999; Juengel et al., 2006), and disruption of these receptors leads to dramatic reproductive phenotypes (Lubahn et al., 1993; Korach et al., 1996). While the precise role for estrogen in promoting cyst breakdown, primordial follicle assembly, and long-term fertility is unknown, it is clearly an essential regulator of this developmental transition.

In order to elucidate fundamental mechanisms associated with healthy primordial follicle assembly, we have examined ovarian development in the context of TAF4b-deficient ovaries during the late embryonic to early postnatal period. While TAF4b-deficient ovaries appear normal during late embryogenesis, they suffer accelerated germ cell death by postnatal day (PND) 1. Excessive germ cell loss in the TAF4b-deficient ovaries is associated with delayed onset of germ cell cyst breakdown compared to control ovaries. Surprisingly, excessive germ cell loss in the TAF4b-deficient ovaries is associated with a significant increase in Activated Caspase 3, activation not normally observed during normal cyst breakdown which is primarily dependent upon Activated Caspase 2 (Bergeron et al., 1998; Morita et al., 2001; Takai et al., 2007; Lobascio et al., 2007; Tingen et al., 2009). Moreover, this form of germ cell attrition in the TAF4b-deficient ovaries can be prevented by apoptosis inhibition and β-estradiol treatment. Together, these studies highlight the critical role of TAF4b in the establishment of the initial and long-term postnatal primordial follicle pool.

Results

TAF4b is required for postnatal oocyte survival immediately after birth

As TAF4b-deficient mice exhibit reduced primordial follicle numbers at PND 21 (Voronina et al., 2007), we aimed to determine the precise timing of their demise. Immuno-staining for germ cell markers Mouse Vasa Homolog (MVH) and Tra98 (Tanaka et al., 1997) within the TAF4b-deficient ovary was performed from late embryogenesis through early postnatal development, and then compared to control ovaries. Ovarian tissue sections obtained from TAF4b-deficient (−/−) or matched heterozygous (+/−) and wildtype (+/+) littermates were stained with antibodies against germ cell markers MVH and Tra98 (Fig. 1A). Heterozygous TAF4b mice are completely fertile and were used as control littermates along with their wild-type counterparts. During late embryogenesis at E18.5, ovaries from all genotypes are largely indistinguishable by germ cell density. In contrast, by PND 1, TAF4b (−/−) mice exhibit excessive and significant Tra98-positive germ cell depletion (Fig. 1B, $p < 0.01$, two-tailed *T*-test). When these

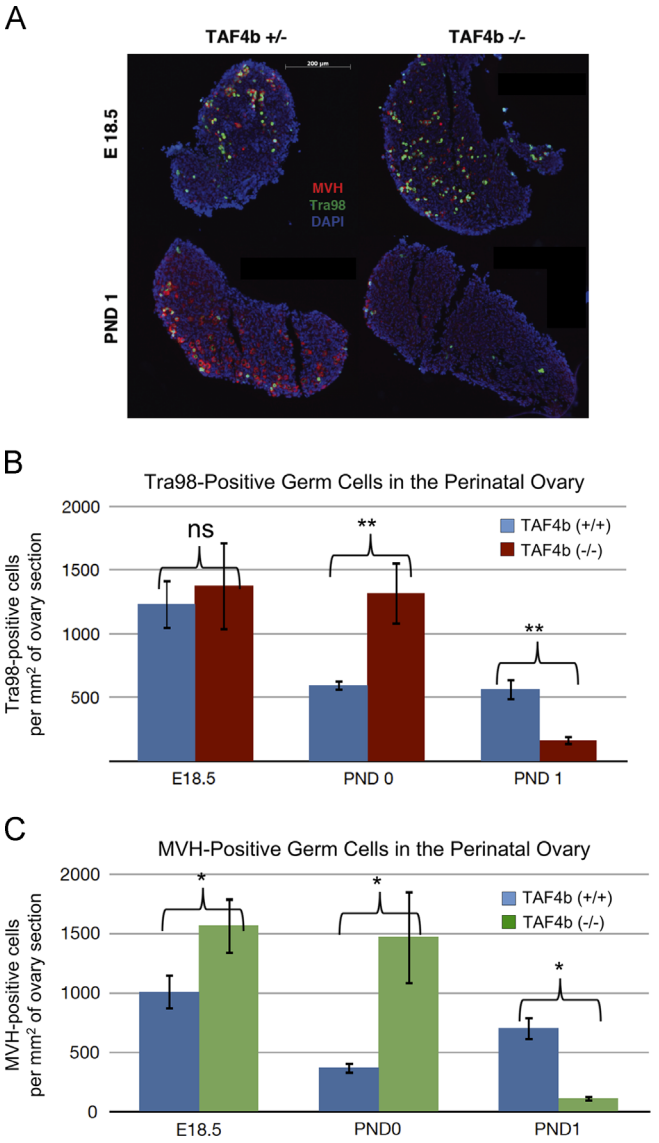

Fig. 1. Perinatal oocyte depletion in TAF4b (−/−) ovaries. (A) MVH (red) and Tra98 (green) staining were used as cytoplasmic and nuclear germ cell markers, respectively, in 8 μm ovary tissue sections. DAPI (blue) denotes nuclei. TAF4b (+/−) and (−/−) ovaries exhibit comparable germ cell densities at E 18.5 (top), while TAF4b (−/−) ovaries suffer excessive depletion of oocyte by PND 1 (bottom). (B) Quantification of stained tissue sections. Tra98-positive oocytes were counted per section and total number was divided by total DAPI area. $N=4$ for PND 0 TAF4b (+/−) and (−/−). $N=5$ for E18.5 TAF4b (−/−). $N=6$ for E18.5 TAF4b (+/−), PND 1 TAF4b (+/−), and PND 1 TAF4b (−/−). **: two-tailed *T*-test, $p < 0.01$. (C) Independent quantification of stained tissue sections. MVH-positive oocytes were counted per section and total number was divided by total DAPI area. $N=3$ for all groups. *: two-tailed *T*-test, $p < 0.05$.