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Unique patterns of Notch1, Notch4 and Jagged1 expression in ovarian vessels during folliculogenesis and corpus luteum formation

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Abstract

Notch signaling functions to regulate cell-fate decisions by modulating differentiation, proliferation, and survival of cells. Notch receptors and ligands are expressed in embryonic vasculature and are required for the remodeling of the primary embryonic vasculature of mice. Here, we characterize the expression patterns of Notch1, Notch4, and Jagged1 proteins during the process of folliculogenesis and corpus luteum formation in the mouse ovary, an organ with dynamic physiological angiogenic growth. These Notch proteins and ligand are expressed in a subset of ovarian vessels, including both mature ovarian vasculature as well as angiogenic neovessels. Their expression in the ovary was found in both endothelial and vascular associated mural cells. Our data suggest a complex regulatory role for the Notch signaling pathway during mouse oogenesis and ovarian neovascularization.

Keywords: Notch1; Notch4; Jagged1; Ovary; Angiogenesis; Follicle; Corpus luteum

1. Results and discussion

The Notch signaling pathway regulates cell-fate determination and patterning of the vascular system. There are four Notch receptors (Notch1-4) and two families of ligands (Jagged1,2 and Delta-like (Dll) 1,3,4), which are also transmembrane proteins. The Notch signaling pathway is initiated by the interaction between extracellular domains of Notch receptors and their ligands on the surface of adjacent cells (Shawber and Kitajewski, 2004; Weinmaster, 1997). Studies have shown that Notch signaling is critical for vascular development. Mice nullizygous for Jagged1, Dll1, Notch1 or Notch1 and Notch4 die in utero on 9.5–10.5 dpc with vascular disorganization and hemorrhaging (Hrabe de Angelis et al., 1997; Krebs et al., 2000; Xue et al., 1999). Expression of an activated form of Notch4 within the developing murine vasculature results in embryonic lethality due to severe vascular abnormalities (Uyttendaele et al., 2001). In the mouse embryo, Notch1, Notch4, Jagged1 and Dll4 are expressed by the endothelial cells of the developing vasculature and become more restricted to arterial endothelial cells as development proceeds (Duarte et al., 2004; Gale et al., 2004; Krebs et al., 2000; Shutter et al., 2000; Uyttendaele et al., 1996; Xue et al., 1999). Notch3 expression is limited to the accessory smooth muscle cells (Villa et al., 2001). Little is known of the expression or function of Notch signaling components in adult vasculature. In this study, we analyzed the expression of Notch proteins Notch1, Notch4 and the Notch ligand, Jagged1, in the mouse ovary during physiological angiogenesis. Previous data demonstrated that Notch2, Notch3 and Jagged2 were expressed in the granulosa cells of developing follicles but not prominently in the vasculature (Johnson et al., 2001). Expression of Jagged1 was also observed in the oocytes.

We first compared the expression of Jagged1, Notch1 and Notch4 in the normal murine ovary to PECAM, an endothelial-expressed protein (Fig. 1). PECAM was expressed in endothelial cells within the theca layer surrounding the follicles (Fig. 1A). A similar staining pattern was found for Jagged1 (Fig. 1B) and Notch4 (Fig. 1D), though cells showed distinctly different levels of

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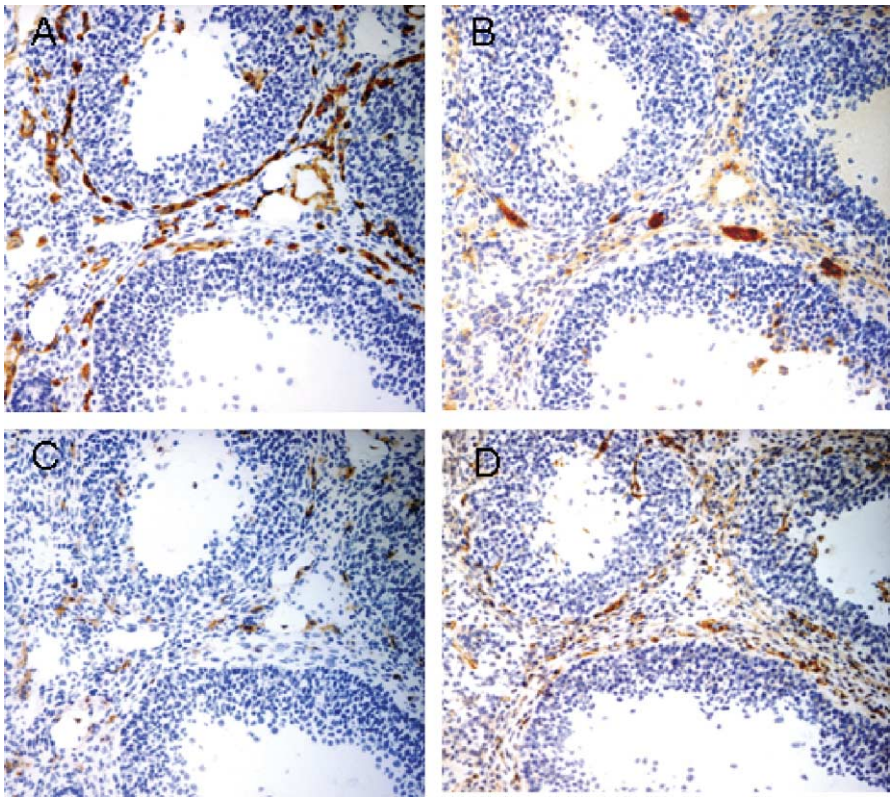

Fig. 1. Normal mouse ovary. 20× magnification. Colormetric IHC. Brown–PECAM, Jagged1, Notch1, Notch4. Blue—nuclei (hematoxylin). (A) Endothelial cells (PECAM). (B) Jagged1. (C) Notch1. (D) Notch4.

expression of these proteins. Notch1 was expressed in a pattern consistent with the ovarian vasculature (Fig. 1C), although only a subset of the endothelial cells surrounding follicles expressed Notch1.

The murine ovary is an organ with dynamic angiogenic growth. Primordial ovarian follicles are avascular structures derived from the mammalian germ cell lineage and make up a 'resting' pool of follicles. The process of folliculogenesis begins when select follicles in the resting pool become preovulatory follicles destined for maturation. Follicle development proceeds to a secondary stage that is characterized by follicles containing centrally located oocytes surrounded by several layers of granulosa cells and a peripheral theca layer containing vasculature (Figs. 2A and 3A). Follicle development to the secondary stage is not dependent on gonadotropins. From this stage onward, mammalian ovaries undergo cyclic changes that are regulated by pituitary hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH). During folliculogenesis, granulosa cells rapidly proliferate around a centrally located, fluid filled cavity referred to as the antrum (Figs. 2B and 3B). Simultaneously, the thecal layer increases in size due to proliferation of both epithelial and endothelial cells. Angiogenesis in the thecal vasculature is required for folliculogenesis to progress (Wulff et al., 2002; Zimmermann et al., 2003, 2001b). At ovulation the follicle ruptures, the granulosa and thecal cells of a ruptured follicle become luteinized and proliferate rapidly to form a solid organ called corpus luteum (Figs. 2C and 3C). As the corpus luteum forms, blood vessels derived from the thecal layer grow into the organ and angiogenesis forms a vascular network (Fraser et al., 2000; Shawber and Kitajewski, 2004; Zimmermann et al., 2001a). In the absence of fertilization and pregnancy, the corpus luteum regresses through apoptotic and non-apoptotic events (Pauli et al., 2005). In contrast, the corpus luteum is maintained for part or all of pregnancy (Fig. 3D). Thus, ovarian folliculogenesis and corpus luteum formation are both characterized by angiogenic development of vascular networks.

To evaluate the expression of Notch genes in the ovary in greater detail, we used a hypophysectomized (HX) mouse model. We have previously established that the HX model replicates normal folliculogenesis and corpus luteum formation of the mouse ovary (Pauli et al., 2005; Zimmermann et al., 2003). In immature HX mice, folliculogenesis and corpus luteum formation is disrupted but can be induced by the administration of gonadotropins (PMSG and HCG). We analyzed ovaries isolated from four groups of mice. *Unstimulated (baseline) group*. HX mice without any gonadotropin stimulation. Ovaries of baseline mice contain primordial (one layer of pregranulosa cells), primary follicles (one layer of granulosa cells) and secondary follicles (more then one layer of granulosa cells and theca layer) (Fig. 2A). The immature follicles of the baseline group