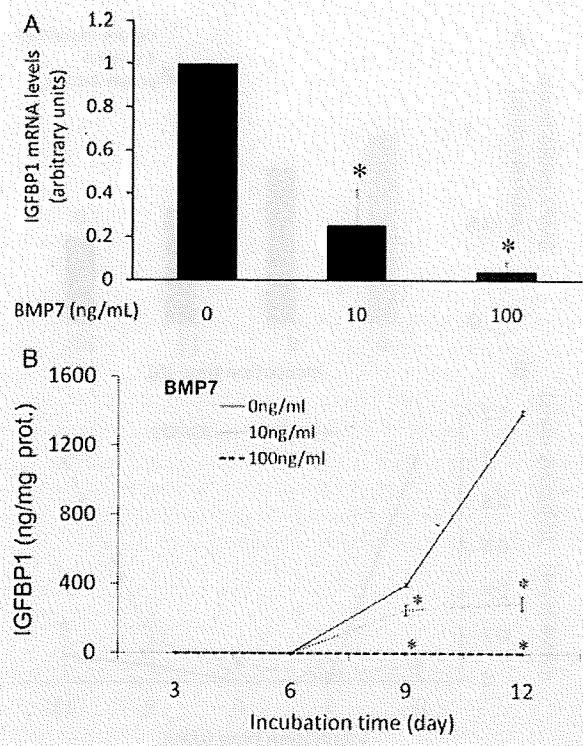

Figure 3 Effects of BMP7 on gene expression and protein secretion of IGFBP1 from ESC. (**A**) Effects of 10 and 100 ng/ml BMP7 on IGFBP1 mRNA expression in ESC treated with a combination of 10 ng/ml estradiol plus 100 ng/ml progesterone (EP) for 12 days. Total RNA isolated from ESC was reverse transcribed and then amplified by real-time PCR using primers for IGFBP1. Values were calculated by subtracting data for signal threshold cycles (Ct) of the internal standard (GAPDH) from Ct values for IGFBP1. Values are the mean ± SEM of four independent experiments using samples from four different patients. $^*P < 0.05$ versus 0 ng/ml. (**B**) IGFBP1 concentrations in culture media of ESC treated with EP, with or without BMP7 (10 and 100 ng/ml), for 3, 6, 9 and 12 days. IGFBP1 concentrations were determined using a specific ELISA and normalized against the total protein of cell lysates from each well. Data are the mean ± SEM of duplicate cultures. $^*P < 0.05$ compared with the respective control on each day. The result is representative of three separate experiments using samples from three different patients.

Discussion

In the present study, we demonstrated that gene expression of BMP7 in the endometrium was lower in the mid- and late secretory phases and in early pregnancy than in the mid-proliferative phase. Progesterone, but not estradiol, decreased BMP7 gene expression in ESC, which was significant after 12 h. Long-term incubation with progesterone and estradiol induced IGFBP1 protein secretion from ESC, which was inhibited by BMP7. BMP7 also decreased ESC proliferation.

In parallel with dynamic changes in the endometrium, the expression of many molecules in the endometrium changes spatiotemporally. Because embryos are accepted by the endometrium only during the 'implantation window', which corresponds to the mid-secretory phase, those substances for which levels in the endometrium change during the mid-secretory phase may have a role in preparing the receptive endometrium. In this context, the decrease in the gene expression of BMP7 in the mid-secretory phase may contribute to the development of the receptive endometrium.

Decidualization is a process in which remarkable structural and functional changes occur in ESC to prepare an appropriate environment for embryo implantation and maintenance of pregnancy. Decidualization is regulated by the ovarian steroid hormones estradiol and progesterone. In addition, the importance of other factors in the induction of decidualization has been demonstrated recently. For example, we found that mechanical stretch augments decidualization (Harada *et al.*, 2006), and others have found that paracrine factors are involved in decidualization (Tang *et al.*, 1994; Fazleabas and Strakova, 2002). The results of the present study, showing that BMP7 suppresses secretion of IGFBP1 protein from decidualizing ESC, suggest that BMP7 may act as an antidecidualization factor in the endometrium.

The antidecidualization activity of BMP7 is in marked contrast with the actions of BMP2, which increases the secretion of IGFBP1 and prolactin, another marker of decidualization, in decidualized ESC (Li *et al.*, 2007; Stoikos *et al.*, 2008). The expression patterns of BMP2 and BMP7 in the endometrium also appear to be different because *in-vitro* decidualization increases the expression of BMP2 in ESC (Li *et al.*, 2007). Thus, as a result of their different spatiotemporal expression, it is possible that the opposing actions of these two BMPs support decidualization and the subsequent establishment of pregnancy. From a therapeutic perspective, therapies targeted for BMP7 and BMP2 could be applicable for the treatment of implantation failure caused by impaired decidualization. Interestingly, the opposing functions of BMP7 and BMP2 have been demonstrated recently in adipogenesis, with BMP7 contributing to the development of brown adipocytes and BMP2 contributing to the development of white adipocytes (Tseng *et al.*, 2008).

The decrease in BMP7 expression in the decidualized endometrium may also be important for the successful development of the placenta. It has been shown that BMP7 suppresses the production of human chorionic gonadotrophin and progesterone from the trophoblast (Martinovic *et al.*, 1996). Because these hormones are tremendously important for the maintenance of pregnancy, the presence of BMP7 in the endometrium would be problematic for invading trophoblasts. Therefore, reduced BMP7 expression may be necessary not only for the development of a receptive endometrium, but also for the invading trophoblasts to establish pregnancy.

Progesterone inhibited BMP7 gene expression in ESC. This suggests that the decreased expression of BMP7 in the endometrium from the mid-secretory phase is due to the effects of progesterone. Notably, the inhibition of BMP7 gene expression by progesterone was clearly observed as early as 12 h. In addition, the decrease in BMP7 expression evidently preceded the increase in IGFBP1 expression during decidualization with progesterone and estradiol. This result, however, appears to be inconsistent with the findings by Stoikos *et al.* (2008) which showed that BMP7 gene expression was not altered by *in vitro* decidualization with cAMP. This difference may indicate that progesterone is prerequisite for down-regulation of BMP7 expression in the process of decidualization. Collectively,

progesterone may suppress BMP7 gene expression in the early stage to facilitate subsequent decidualization. Another apparently inconsistent finding of Stoikos *et al.* (2008) was the vesicular staining for BMP7 in decidual cells in mid–late secretory endometrium although staining patterns were not shown in other phases of the menstrual cycle. The decrease in BMP7 gene expression by progesterone might be involved in the change, if any, of intracellular localization of BMP7. Another possible explanation for the inconsistency may be any cross-reactivity of the antibody used in that study.

BMP7 appears to stimulate or inhibit proliferation depending on the cell type; for example, BMP7 stimulates proliferation of ovarian granulosa cells (Lee *et al.*, 2001) and Sertoli cells (Puglisi *et al.*, 2004), but inhibits proliferation of aortic smooth muscle cells (Dorai *et al.*, 2000), renal mesangial cells (Otani *et al.*, 2007) and prostate cancer cells (Miyazaki *et al.*, 2004). In the present study, BMP7 inhibited the proliferation of ESC. Thus, the decrease in BMP7 expression in the decidualized endometrium may contribute to the proliferation of decidual cells during pregnancy.

The present study has some limitations. First, the decidual tissues of ectopic pregnancies used in this study have advantages in that they are free from contamination with trophoblast cells, but they may have different characteristics from deciduas of normal pregnancies. Second, we measured mRNA levels but not protein levels of BMP7. Although cellular protein levels shown by immunostaining or immunoblotting are not necessarily proportional to their functional activities, knowledge about them would help our understanding of BMP7 in the endometrium. A further study is warranted regarding this point.

In summary, the results of the present study suggest that progesterone decreases BMP7 expression in the endometrium. The decrease in BMP7 expression may facilitate decidulalization of the endometrium, thus aiding the establishment of pregnancy.

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