

Expression and hormone regulation of Wnt2, 3, 4, 5a, 7a, 7b and 10b in normal human endometrium and endometrial carcinoma

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Summary Wnt genes are transforming to mouse breast epithelium and are hormonally regulated in vivo. To assess their role in another endocrine-responsive human cancer, the expression of seven Wnt genes (Wnt 2, 3, 4, 5a, 7a, 7b and 10b) in normal human endometrium and endometrial cells, and endometrial carcinoma tissues and cell lines was investigated by ribonuclease protection analysis. Wnt2, 3, 4 and 5a mRNAs but not Wnt7a, 7b or 10b mRNAs were expressed in primary culture of normal endometrial epithelial (NEE) and stromal (NES) cells. In contrast, in four endometrial carcinoma cell lines (RL95-2, HEC-1-A, AN3 CA and Ishikawa), Wnt2 and Wnt3 mRNAs were absent. Wnt4 was expressed in only one out of four cell lines (RL95-2), and Wnt5a was much lower. Wnt7a and Wnt7b mRNAs were expressed in three out of four cell lines (RL95-2, HEC-1-A and Ishikawa). Wnt10b mRNA was expressed in RL95-2 and AN3 CA. In fresh tissues, all Wnt genes apart from Wnt10b were expressed in normal endometrium and endometrial carcinoma. Similar to the cell lines, the level of Wnt4 mRNA expression was significantly higher in the normal endometrium than endometrial carcinoma. Wnt2, 3 and 5a mRNAs were also lower in endometrial carcinoma compared with normal endometrium. There was no difference in the level of Wnt2, 3, 4 and 5a mRNA expression between proliferative phase and secretory phase of the menstrual cycle, or between either menstrual phase and the first trimester of pregnancy. In vitro, progesterone and/or 17 β -oestradiol had no effect on Wnt2, 3, 4, 5a and 7b mRNA expression in NES and all endometrial carcinoma cell lines. The data indicate that all Wnt genes were expressed in vitro, six out of seven Wnt genes (Wnt 2, 3, 4, 5a, 7a and 7b) were expressed endogenously in the human endometrium, their mRNA expression was hormonally independent and Wnt4 gene down-regulation as well as down-regulation of Wnt 2, 3 and 5a may be associated with endometrial carcinoma.

Keywords: endometrial cancer; endometrium; gene expression; Wnt gene

Wnt genes make up a large family of highly conserved developmental genes. The first member, *int-1*, was discovered as a common integration site of mouse mammary tumour virus (MMTV) in mammary epithelial adenocarcinomas (Nusse and Varmus, 1992). *Int-1* exhibits a high homology to the *Drosophila* developmental gene *wingless* that is involved in pattern formation. The combination of *wingless* and *int-1* gives rise to the term Wnt so that *int-1* became Wnt1 and is the first member of the Wnt gene family (Nusse et al, 1991). In the MMTV-induced mouse mammary carcinoma, two additional *Wnt3* and *Wnt10b* genes are also known to be activated concomitantly with *FGF3* and *FGF8* genes, respectively, indicating members of the Wnt and FGF families act co-operatively to induce tumorigenesis (Roelink et al, 1990; Lee et al, 1995). On its own, *Wnt1* is capable of inducing mammary hyperplasia and carcinoma that are unaffected by ovariectomy and adrenalectomy in both transgenic male and female mice (Lin et al, 1992; Edwards et al, 1992). In the presence of *FGF3*, the rate of *Wnt1*-induced mouse mammary hyperplasia is increased indicating the role of FGF in accelerating tumorigenesis

(Kwan et al, 1992). Evidence from the in vitro studies has also demonstrated the ability of some Wnt genes (*Wnt1*, 2, 3a, 5b, 7a and 7b) to cause partial transformation in the mouse mammary epithelial cell line, C57MG (Wong et al, 1994) and *Wnt1*, 6 and 7b in the mouse embryonic fibroblast cell line, C3H 10T1/2 (Bradbury et al, 1994). A subset of the murine Wnt genes (*Wnt2*, 4, 5a, 5b, 6 and 7b) has been found to be expressed differentially in virginal, pregnant, lactating and involuting mammary tissues (Gavin et al, 1992; Buhler et al, 1993; Weber-Hall et al, 1994). Furthermore, *Wnt2*, *Wnt4* and *Wnt5b* are regulated by ovarian hormones indicating the role of Wnts in the normal development of the mouse mammary gland.

Homologues of mouse Wnt genes have been isolated in *Drosophila*, *Xenopus*, chicken and humans (Nusse and Varmus, 1992). In humans, there are nine Wnt genes known [*Wnt1* (van Ooyen et al, 1985), *Wnt2* (Wainright et al, 1988), *Wnt3* (Roelink et al, 1993), *Wnt5a* (Clark et al, 1993; Lejeune et al, 1995), *Wnt3a*, *Wnt4*, *Wnt7a* and *Wnt7b* (Huguet et al, 1994), and *Wnt10b* (Bui et al, 1997a)]. Four Wnt genes (*Wnt2*, 4, 5a and 7b) are more highly expressed in human breast carcinomas compared with normal breast tissues (Huguet et al, 1994; Lejeune et al, 1995). Additionally, *Wnt5a* is also up-regulated in lung, colon and prostate carcinomas and melanomas (Iozzo et al, 1995), *Wnt2* is up-regulated in colon carcinomas (Vider et al, 1996) and *Wnt7b* is

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up-regulated in superficial bladder carcinomas compared with normal bladder tissues and invasive bladder carcinomas (Bui et al, 1997b). This accumulating evidence supports the role of *Wnt* genes in the development of human malignancy.

In the mouse uterus, *Wnt5a* is expressed in the uterine mesenchyme but not in the uterine or vaginal epithelium, and is required for the induction of the homeobox-containing gene, *Msx1*, in Mullerian epithelium. The *Msx1* gene, in turn, plays an important role in maintaining the adult uterus in a morphogenetic and developmentally responsive state (Pavlova et al, 1994). The expression of *Wnt* genes in the human uterus has not yet been reported. Therefore, the aim of this study was to examine the expression of seven *Wnt* genes in normal human endometrium, primary cultures of endometrial cells, endometrial carcinoma tissues and cell lines in order to evaluate hormonal regulation in the human endometrium and differential expression in endometrial cancer.

MATERIALS AND METHODS

Primary cells, cell lines, tissue samples and RNA preparation

The primary normal human endometrial epithelial (NEE) and stromal (NES) cells were isolated and maintained as described (Zhang et al, 1995). The human endometrial carcinoma cell lines were obtained from the American Type Culture Collection, Bethesda, MD, USA: RL95-2 (CRL 1671), HEC-1-A (HTB 112) and AN3 CA (HTB 111); and Ishikawa from Dr John White (Hammersmith Hospital, London, UK). The normal human endometrium and endometrial carcinoma samples were obtained at hysterectomy, frozen immediately and stored in liquid nitrogen until required. The stage of the menstrual cycle of the tissue was determined from the patient's menstrual history and endometrial histology (Noyes et al, 1950; Ferenczy, 1987; Buckley and Fox, 1989). Human first trimester decidua was obtained at termination of pregnancy and stored in liquid nitrogen until required. All the cells were cultured in Dulbecco's modified Eagle medium (DMEM) (Imperial Cancer Research Fund Clare Hall Laboratories, UK) and 10% fetal calf serum (FCS; Gibco), on plastic culture plates (Becton Dickinson) at 37°C, 5% carbon dioxide/95% air, in a humidified incubator. The cells were allowed to reach confluence before harvest. Total RNA was prepared from tissues and cells using the acid guanidium thiocyanate-phenol-chloroform extraction method as described (Chomczynski and Sacchi, 1987), followed by a 5.7 M caesium chloride separation in polyallomer tubes (13 × 51 mm; Beckman) at 50 000 r.p.m. for 3 h using SW50 or SW55 swing rotor (Beckman) in the L8-80M ultracentrifuge (Beckman). The RNA pellet was resuspended in 200 µl of sterile water, treated with RNAase-free DNAase at 37°C for 15 min, extracted with an equal volume of phenol, ethanol precipitated with 0.1 × volume of sodium acetate, pH 5.2, and resuspended in water to the final concentration of 1 µg µl⁻¹.

Treatment of cells with progesterone and 17β-oestradiol

Cells were grown to confluence and then allowed to quiesce for 1 week in oestrogen-free medium containing phenol red-free DMEM/10% dextran-coated charcoal-stripped FCS. Cells were then treated with fresh oestrogen-free medium containing either 5 × 10⁻⁹ M progesterone (Sigma) or 5 × 10⁻¹⁰ M 17β-oestradiol (Sigma) for 18 h. Total RNA was harvested from cells as described above.

Riboprobe constructs and ribonuclease (RNAase) protection analysis

The human *Wnt* 2, 3, 4, 7a and 7b (Huguet et al, 1994), *Wnt5a* (Lejeune et al, 1995), *Wnt* 10b (Bui et al, 1997b) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (McCarthy and Bicknell, 1992) riboprobe constructs have been described. The linearized plasmid DNA was labelled with α[³²P]CTP (Amersham) to generate antisense riboprobe, which was then purified using the Spin Column according to the manufacturer's instructions (Boehringer Mannheim). RNAase protection analysis was performed using standard protocols (Ausubel et al, 1990). In brief, 10 µg of total RNA was hybridized to 30 µl of hybridization mix containing 10⁵ c.p.m. of *Wnt* and 5.0 × 10⁴ c.p.m. of GAPDH antisense riboprobes at 45°C for 5–12 h. After RNA-RNA hybridization, RNAase digestion with RNAaseA and RNAaseT₁ was performed at room temperature for 30 min, followed by treatment with protein K at 37°C for 15 min. The sample was then extracted with an equal volume of phenol, precipitated with 0.1 × volume of sodium acetate, pH 5.2, electrophoresed on a 6% polyacrylamide/urea gel and autoradiographed at -70°C with intensifying screens. Yeast total RNA (Boehringer Mannheim) was used as a negative control. The protected fragment signals for *Wnt* and GAPDH were quantified by laser densitometry using a Bio Image analyser (Millipore). The level of *Wnt* mRNA expression was shown as a ratio of *Wnt*/GAPDH protected fragment signals.

Statistical analysis

The level of expression of all the *Wnts* in human endometrial tissues and endometrial tumours were compared using the Student unpaired *t*-test, using the Minitab version 8.2.

RESULTS

The expression of *Wnt* mRNAs in normal human endometrial cells and endometrium, and endometrial carcinoma cell lines and endometrial tumours was determined by RNAase protection analysis.

Expression of *Wnt* mRNAs in normal human endometrial cells and endometrial cells and endometrial carcinoma cell lines

Table 1 summarizes *Wnt* mRNA expression in normal endometrial epithelial (NEE) and stromal (NES) cells, and four endometrial carcinoma cell lines: RL95-2, HEC-1-A, AN3 CA and Ishikawa. The value indicates the ratio of *Wnt* gene expression to GAPDH gene expression, where zero indicates no detectable *Wnt* protected fragment after 7 days' exposure. *Wnt*2, 3 and 4 mRNAs were expressed in normal endometrial cells but not endometrial carcinoma cell lines, apart from *Wnt*4 in RL95-2. *Wnt*5a mRNA was highly expressed in normal endometrial cells but low or even absent in endometrial carcinoma cell lines. *Wnt*7a, 7b and 10b mRNAs were absent in normal endometrial cells but expressed at a varying degree in endometrial carcinoma cell lines. In the normal endometrial cells, NES expressed higher levels of *Wnt*2, 4 and 5a mRNAs than NEE. *Wnt*5a was highly expressed in NEE and NES cells, at least twofold higher than GAPDH (result not shown). In all assays, the negative control tRNA yielded no RNA protected

Table 1 Expression profile of Wnt mRNAs in normal human endometrial cells and endometrial carcinoma cell lines as determined by RNAase protection analysis

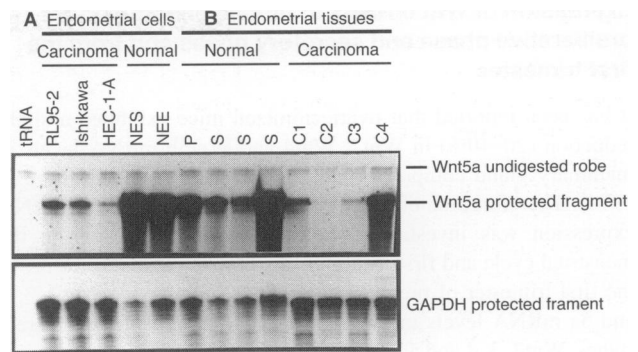
Endometrial cells	Wnt	2	3	4	5a	7a	7b	10b
RL95-2	ER -ve PR +ve	0	0	1.6	0.2	5.9	4.6	2.0
HEC-1-A	ER -ve PR +ve	0	0	0	0.1	5.7	0.4	0
AN3 CA	ER -ve PR +ve	0	0	0	0	0	0	1.3
Ishikawa	ER +ve PR+ve	0	0	0	0.2	0.9	0.3	0
NEE	ER +ve PR +ve	0.5	2.4	9.8	2.2	0	0	0
NES	ER +ve PR +ve	18.8	3.0	21.5	9.4	0	0	0

Quantified level of Wnt mRNA expression was shown as a ratio of the optical density value of Wnt protected fragment signal to the optical density value of GAPDH protected fragment signal of the same sample carried out in the same assay. The results of each Wnt were obtained from the same experiment so that a direct comparison could be made between cell lines. ER, oestrogen receptor; PR, progesterone receptor (Zhang et al, 1995).

fragment. A representative autoradiograph (Figure 1A) shows a specific Wnt5a mRNA protected fragment and corresponding GAPDH mRNA protected fragment in endometrial cells. The data show that Wnt2, 3, 4 and 5a mRNAs were expressed at higher levels in normal endometrial cells than endometrial carcinoma cell lines; whereas Wnt7a, 7b and 10b mRNAs were absent in normal endometrial cells but expressed in some endometrial carcinoma cell lines.

Expression of Wnt mRNAs in human endometrium and endometrial carcinoma

The Wnt mRNA expression was then assessed in intact human endometria and endometrial tumours. Four normal human endometrial samples were from the proliferative phase (P) and seven from the secretory phase (S). Four human endometrial carcinomas were at the superficial stage I and grade II of the disease in which the tumour was confined within the uterus. Table 2 summarizes Wnt mRNA expression in the normal human endometrial tissues and endometrial carcinomas. The value indicates the ratio

**Figure 1** RNAase protection analysis of Wnt5a mRNA expression in human endometrial cells (A) and tissues (B). tRNA is a negative control. P, proliferative phase; S, secretory phase; C, carcinoma

of Wnt gene expression to GAPDH gene expression, where zero indicates no detectable Wnt protected fragment after 7 days' exposure. Wnt2, 3, 4 and 5a mRNAs were expressed at higher levels in normal endometrial tissues than endometrial carcinomas. There was a statistically significant difference of Wnt4 mRNA expression between normal endometrial tissues and endometrial carcinomas ($P = 0.03$).

Since there were not enough normal endometrial samples, the expression of Wnt7a and Wnt7b is shown for the individual cases. The same normal endometrial tissues were used to analyse Wnt7a and Wnt7b. One normal endometrial tissue obtained at the secretory phase of the menstrual cycle produced a very strong Wnt7a expression. The same sample also produced a detectable protected fragment for Wnt7b. In the endometrial carcinoma, Wnt7a and Wnt7b showed a wide range of expression overlapping with the normal endometrium. One endometrial carcinoma sample (C4) consistently expressed a higher level of Wnt2, 3, 4, 5a and 7a compared with the other three endometrial carcinoma samples (C1–C3). Wnt10b was not detected in normal endometrial tissues and endometrial carcinomas after 7 days' exposure.

Figure 1B is a representative autoradiograph showing a specific Wnt5a mRNA protected fragment and corresponding GAPDH mRNA protected fragment in human endometrial tissues. The data show that Wnt2, 3, 4 and 5a exhibited a similar pattern of high mRNA expression in normal endometrial tissues and low mRNA expression in endometrial carcinomas.

Table 2 Quantified levels of Wnt mRNA expression in human endometria and endometrial carcinomas by RNAase protection analysis

Endometrial tissues	Wnt2	Wnt3	Wnt4	Wnt5a	Wnt7a	Wnt7b	Wnt10b
Proliferative (P) (n = 4)	Median = 73 Range 54–433	Median = 33 Range 29–52	Median = 27 Range 8–68	Median = 110.5 Range 62–279	n = 1 2	n = 1 15	0
Secretory (S) (n = 7)	Median = 44 Range 20–170	Median = 29 Range 11–110	Median = 40 Range 29–77	Median = 65 Range 5–156	n = 3 33, 91, 1058	n = 3 0, 0, 3	
Carcinoma (C1)	5	3	0	3	123	98	0
Carcinoma (C2)	4	4	0	1	51	8	0
Carcinoma (C3)	2	8	0	3	4	0	0
Carcinoma (C4)	116	21	3	13	214	0	0

The Wnt mRNA expression values were obtained as a ratio of the optical density value of Wnt protected fragment signal to the optical density value of GAPDH protected fragment signal of the same sample carried out in the same assay. P, S and C refer to proliferative phase, secretory phase and carcinoma respectively.

Expression of Wnt mRNAs in human endometria at proliferative phase and secretory phase and from the first trimester

It has been reported that ovariectomized mice exhibited a slight reduction (20–40%) in Wnt2, Wnt4 and Wnt5b mRNA levels in mammary gland compared with control mice (Weber-Hall et al, 1994). Therefore, the effect of ovarian hormones on Wnt mRNA expression was investigated in human endometria during the menstrual cycle and first trimester. Six human endometria were in the first trimester of pregnancy. Figure 2 summarizes Wnt2, 3, 4 and 5a mRNA levels in the human endometria at three different stages. Wnt2, 3, 4 and 5a were strongly expressed in all the tissues. Wnt7a and Wnt7b were expressed at a low level. Wnt10b was not expressed. Figure 3 is a representative autoradiograph showing a specific Wnt4 mRNA protected fragment and corresponding GAPDH mRNA protected fragment in human endometria at three different stages. Statistically, there was no significant difference in

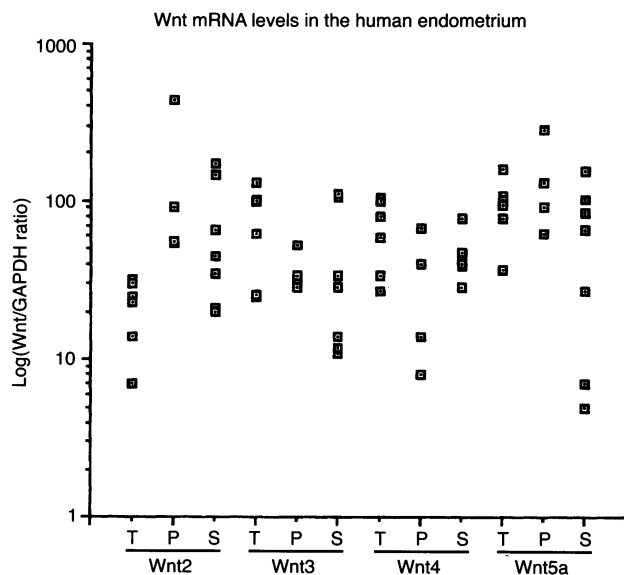


Figure 2 Quantified levels of Wnt mRNA expression in human endometria at proliferative phase (P), secretory phase (S) and first trimester (T)



Figure 3 RNAse protection analysis of Wnt4 mRNA expression in human endometria at proliferative phase (P), secretory phase (S) and first trimester (T). tRNA is a negative control

Wnt2, 3, 4 and 5a mRNA expression between the proliferative phase and secretory phase of the menstrual cycle, or between either the menstrual phase and first trimester (result not shown). The data show that *in vivo* ovarian hormones had no effect on the Wnt mRNA expression investigated.

Hormonal effect on Wnt mRNA expression in vitro in NES and endometrial carcinoma cell lines

The effect of ovarian hormones on Wnt mRNA expression was also investigated *in vitro*. After 18 h hormonal treatment at a physiological concentration of progesterone or oestrogen, there was no difference in the levels of Wnt2, 3, 4, 5a and 7b mRNAs between control and progesterone and/or 17 β -oestradiol-treated cells. However, mRNA for vascular endothelial growth factor and midkine was also studied and was induced at 18 h (Zhang et al, 1995). Figure 4 is a representative autoradiograph showing a specific Wnt5a mRNA protected fragment and corresponding GAPDH mRNA protected fragment in control and hormonal-treated cells. The data show that progesterone and 17 β -oestradiol had no effect on the Wnt mRNA expression investigated *in vitro*, although other hormonal-regulated genes responded.

DISCUSSION

There is emerging evidence indicating that *Wnt* genes may play a role in the genesis of human malignancy, and different *Wnt* genes are involved in different tumour types (Huguet et al, 1994; Iozzo et al, 1995; Lejeune et al, 1995; Vider et al, 1996). We analysed Wnt mRNA expression in human endometrium and endometrial carcinoma cell lines derived from and in fresh tissues.

The level of Wnt4 mRNA expression was significantly higher in normal endometrium than endometrial carcinoma, suggesting that Wnt4 down-regulation might be important in the development of endometrial cancer. This down-regulation of Wnt4 mirrors that seen following morphological transformation of C57MG cells induced by Wnt1 or Wnt2 or activated *neu* tyrosine kinase receptor (Olson and Papkoff, 1994). Three out of the four endometrial carcinoma samples also expressed lower Wnt2, 3 and 5a mRNA levels compared with normal endometrium, suggesting

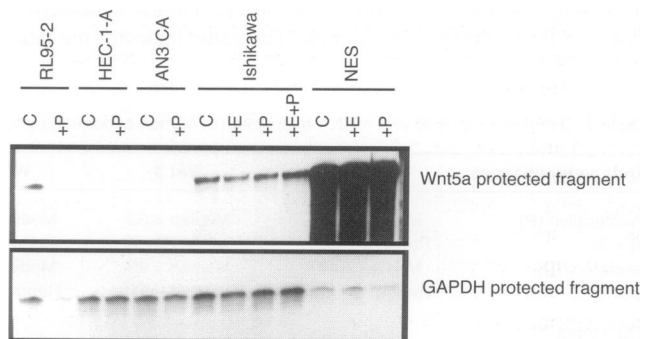


Figure 4 RNAse protection analysis of Wnt5a mRNA expression in control and hormone-treated endometrial cells. Total RNA (5 μ g) was used in NES, whereas 10 μ g of total RNA was used in endometrial carcinoma cell lines. C, untreated; +, treated for 18 h; E, 5 $\times 10^{-10}$ M 17 β -oestradiol; P, 5 $\times 10^{-9}$ M progesterone

these genes may also have a role in normal differentiation. In all the known differentially expressed *Wnts*, this is the first case in which a *Wnt* gene is down-regulated by at least sixfold in human tumour tissues compared with normal tissues.

It was possible that the difference in Wnt mRNA expression between normal endometrium and endometrial carcinoma was caused by different responses to ovarian hormones. This has been demonstrated in the mouse mammary gland in which *Wnt2*, 4 and 5b were slightly down-regulated by ovarian hormones (Weber-Hall et al, 1994). The data obtained from the endometria in the proliferative phase and secretory phase of the menstrual cycle and in the first trimester of pregnancy in which the levels of oestrogen and progesterone vary dramatically, showed no variation in mRNA expression of *Wnt2*, 3, 4 and 5a. Additionally, the in vitro data show that hormonal treatment had no effect on the mRNA expression of these *Wnt* genes plus *Wnt7b*.

Since the endometrium consists of a mixture of different cell populations, largely epithelia and stroma, it is possible that the level of Wnt mRNA expression might relate to the ratio of epithelia to stroma, as is seen in the mouse uterus in which *Wnt5a* is expressed endogenously in the uterine mesenchyme, which then acts on the neighbouring uterine epithelia to induce expression of a homeobox-containing gene, *Msx-1*, for uterine development (Pavlova et al, 1994). Using highly homogeneous isolated primary normal endometrial epithelial and stromal cells (Zhang et al, 1995), *Wnt2*, 3, 4 and 5a mRNAs were shown to be expressed more highly in the normal human endometrial cultures, NES and NEE, compared with endometrial carcinoma cell lines. The expression of *Wnt2*, 3, 4 and 5a genes in turn reflected the Wnt expression seen in vivo. Therefore, the predominant cell types isolated from normal endometrium exhibited a different phenotype from the tumours. However, it could not be excluded that a minor population of normal endometrial cells also had the same phenotype as the tumours, which could give rise to the differential *Wnt2*, 3, 4 and 5a expression between normal endometrial cells and endometrial carcinoma cell lines.

In vitro, *Wnt7a* and *Wnt7b* were detected in endometrial carcinoma cell lines but not in normal endometrial cultures. In comparison with in vivo, both *Wnt7a* and *Wnt7b* were detected in normal endometrial tissues and endometrial tumours. This discrepancy of *Wnt7a* and *Wnt7b* expression in the normal endometrial cultures and tissues could be caused by the fact that NEE and NES were cultured cells and were maintained in an artificial environment, and regulatory signals that determine *Wnt7a* and *Wnt7b* expression were removed. Therefore, NES and NEE may provide good models for studying *Wnt* gene regulation and the factors that perturb this normal Wnt mRNA expression pattern. The levels of *Wnt2*, 4 and 5a mRNA were higher in stromal cells than epithelial cells, whereas the level of *Wnt3* mRNA was approximately equal, suggesting the potential role of *Wnt2*, 4 and 5a in cell signalling between stroma and epithelia.

In comparison with normal endometrial cells, *Wnt2*, 3, 4 and 5a mRNAs were either absent or expressed at a very low level in endometrial carcinoma cell lines. These results indicate that the down-regulation of *Wnt2*, 3, 4 and 5a mRNA expression may be associated with endometrial neoplasia. It has been demonstrated that lowering of *Wnt5a* mRNA level in vitro will increase cell branching that resembles cell migration (Huguet et al, 1995). Therefore, the low levels of *Wnt5a* mRNA expression in endometrial carcinoma cell lines and tissues were in agreement with the

role of *Wnt5a* as a modulator of cell migration (Moon et al, 1993). This view is further strengthened by the observation that the level of *Wnt5a* mRNA was extremely high in NES and NEE, and was even higher than the GAPDH mRNA level.

Wnts are a group of novel growth factors that act in an autocrine and/or paracrine manner to affect cell signalling via the cell adhesion molecules (Bradley et al, 1993; Hinck et al, 1994). Little is known about the interaction of different *Wnt* members or the effect of epithelia–stroma interaction of Wnt mRNA expression. The normal endometrial cells used in this study may be a useful model for addressing these questions. In conclusion, the results presented here indicate that a subset of the human *Wnt* genes (*Wnt2*, 3, 4 and 5a) exhibited a common differential pattern of mRNA expression between normal and malignancy of the endometrium both in vitro and in vivo. Six *Wnt* genes (*Wnt2*, 3, 4, 5a, 7a and 7b) were expressed endogenously in the human endometrium, their mRNA expression was hormonally independent and *Wnt4*, as well as *Wnt2*, 3 and 5a, gene down-regulation may be associated with endometrial carcinoma. The effect of down-regulation of *Wnt4* has been produced by transforming *Wnt1* or *Wnt2* in the murine mammary epithelial cell line C57MG. We are therefore assessing whether as yet unidentified overexpressed *Wnt* is inducing this effect.

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