Differential expression of the PEA3 subfamily of ETS transcription factors in the mouse ovary and peri-implantation uterus

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Abstract

The objective of the present investigation was to examine the spatio-temporal expression of three members of the ETS family of transcription factors, ERM, ER81, and PEA3, in the peri-implantation mouse uterus and in the ovary. These three factors belong to the PEA3 subfamily and are known to mediate diverse functions ranging from neuronal development to tumor progression. As transcription factors, they regulate the expression of a number of genes with various biological functions. Since several genes with known roles in the reproductive processes have been shown to be under the regulation of one of these factors, we sought to investigate the expression of ERM, ER81, and PEA3 in the mouse ovary and uterus. Quantitative RT-PCR analyses showed that ERM, ER81, and PEA3 were all expressed in the peri-implantation mouse uterus, with higher levels of expression on days 4 and 5 of pregnancy. To determine the cell type-specific expression of these factors, we employed *in situ* hybridization, the results of which revealed that ERM was expressed in both the epithelium and the stroma on days 4 and 5 of pregnancy. Uterine glands showed a high expression of ERM on those days. ERM was also highly expressed in the corpora lutea of the mouse ovary. Both ER81 and PEA3 were expressed at low levels in the stroma on days 4 and 5. On day 8, while ERM and PEA3 were mainly expressed in the embryo and were at low levels in the maternal decidua in a diffused pattern, ER81 was highly expressed in the vascular bed of the mesometrial deciduum. Both ER81 and PEA3 were undetectable in the mouse ovary. Collectively, these data show that ERM is implicated in the early event of implantation as well as in ovarian functions, while ER81 is involved in the establishment of the maternal vasculature for subsequent placental development. PEA3 is apparently an embryonic factor for early embryogenesis.

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Introduction

Embryo implantation is a critical event regulating successful pregnancy. It is a complex process involving intricate interactions between the blastocyst and the uterus. During this process, the uterus undergoes morphological and physiological changes in transforming from a non-receptive to a receptive state (reviewed by Dey 1996). The main driving force for this transformation is the ovarian hormones which act through their intracellular receptors to regulate gene expression and hence influence cellular proliferation and differentiation. The uterus consists of three major cell types, the epithelium, stroma, and myometrium. In mice, the presence of a vaginal plug after

mating defines day 1 of pregnancy, and the uterus on this day is under estrogenic influence due to a preovulatory estrogen surge, driving proliferation of the epithelium. From day 3 onward, newly formed corpora lutea in the ovaries secrete progesterone, a major hormone of pregnancy, and this hormone induces stromal cell proliferation. With a small amount of estrogen produced on the morning of day 4 of pregnancy, progesterone prepares the uterus for the attachment reaction that occurs around midnight of day 4 in mice. Following the attachment of the blastocyst onto the uterine epithelium, stromal cells proliferate and differentiate into decidual cells to support the developing conceptus under progesterone (reviewed by Dey 1996, Dey *et al.* 2004). In the uterus, a unique set of

genes is expressed in a timely manner during the peri-implantation period for the establishment of uterine receptivity and attachment reaction (reviewed by Dey *et al.* 2004).

The ETS (E26 transformation specific) family of transcription factors is divided into several subfamilies based mainly on sequence homology and location of the ETS domain, an 84 amino acid sequence present in all members of the family (Sharrocks 2001). The PEA3 subfamily is composed of three highly homologous factors, ERM (Etv5), ER81 (Etv1), and PEA3 (Etv4). ETS factors are capable of regulating transcription by binding to ~10 bp elements in the promoters of target genes, known as ETS-binding sites (EBS; 5'-GGA(A/T)-3'). Individual ETS proteins demonstrate specificity for sequences flanking this core, but it is not uncommon for different ETS factors to bind to the same EBS. All three members of the PEA3 subfamily commonly activate transcription. ERM and PEA3 are also known to be downstream effectors of fibroblast growth factor (FGF) signaling (Roehl & Nusslein-Volhard 2001) and, in several developmental contexts, ERM and PEA3 are present in regions of FGF signaling (Brent & Tabin 2004).

ETS factors have been linked to diverse biological processes, but no clear unifying theme has emerged. Gene targeting experiments have provided clues about the function of the members of the PEA3 subfamily. PEA3-deficient male mice exhibit ejaculatory dysfunction possibly stemming from neuronal defects (Laing *et al.* 2000). ER81-deficient mice exhibit severe motor discoordination and die around 3–5 weeks of age (Arber *et al.* 2000). These findings thus suggest that the PEA3 subfamily is involved in neuronal pathfinding. Other members of the ETS family are involved in angiogenesis, immune functions, and various developmental processes (Sharrocks 2001). However, it is not known if these transcription factors are expressed in the uterus during early pregnancy. Since PEA3 members are shown to be involved in regulating several genes relevant to reproductive functions, such as cyclo-oxygenase-2 (COX-2) and proteases (Rorth *et al.* 1990, Crawford *et al.* 2001, Howe *et al.* 2001, Levallet *et al.* 2001, El-Tanani *et al.* 2004), we sought to examine the spatio-temporal expression of ERM, ER81, and PEA3 in the mouse ovary and peri-implantation uterus by quantitative RT-PCR (qRT-PCR) and *in situ* hybridization. Our results have shown that these genes exhibit a distinct expression pattern in these tissues, suggesting diverse roles of these factors in ovarian and uterine functions.

Materials and Methods

Mice and tissue preparation

Adult CD-1 mice (Charles River Laboratories, Wilmington, MA, USA) were housed in the Animal Care Facility at the Washington University School of Medicine according to NIH and institutional guidelines for laboratory animals.

Ovaries were collected from cycling adult mice. For timed pregnancy, female mice were mated with fertile males of the same strain to induce pregnancy. The morning of finding a vaginal plug was designated day 1 of pregnancy. Mice on days 1, 4, 5, and 8 of pregnancy were killed at 0900 h, and their uteri were collected for RNA preparation and *in situ* hybridization. Pregnancy on days 1 or 4 was confirmed by recovering embryos from the reproductive tracts. Implantation sites on day 5 were visualized by intravenous injection (0.1 ml/mouse) of Chicago Blue dye solution (1% in saline) and were cut with a sharp scalpel. Day 8 implantation sites containing decidua and embryos were surgically separated from myometrium for RNA preparation.

qRT-PCR

Total RNA was purified using TRI-Reagent (Sigma-Aldrich, St Louis, MO, USA) according to the manufacturer's protocol. Each uterine RNA sample was collected from a single mouse ($n = 4$ or 5 per group). One microgram of RNA was subjected to RT using M-MuLV reverse transcriptase (Roche Applied Science, Indianapolis, IN, USA) for cDNA synthesis. qRT-PCR (qRT-PCR) was performed by monitoring in real time the increase in fluorescence of the SYBR Green dye (Molecular Probes, Eugene, OR, USA) as described (Wittwer *et al.* 1997, Morrison *et al.* 1998) using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). For comparison of transcript levels between samples, a standard curve of cycle thresholds for several serial dilutions of a cDNA sample was established and then used to calculate the relative abundance of each gene. Values were then normalized to the relative amounts of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA, which were obtained from a similar standard curve. All PCR reactions were performed in duplicate. Sequences of primers used for PCR analysis are given in Table 1.

Hybridization probes

Suitable regions from full-length mouse ERM, ER81, and PEA3 sequences were amplified by PCR and subcloned into pGEM-T Easy Vector (Promega, Madison, WI, USA). Primers used for subcloning are shown in Table 1. Mouse ERM subclone contains nucleotides 826–1048 of NM_023794. ER81 and PEA3 subclones contain 418 bp and 531 bp sequences from untranslated regions respectively. Antisense ³⁵S-labeled cRNA probes were generated using appropriate RNA polymerases. The probes were used at specific activities of 2×10^9 d.p.m./ml.

In situ hybridization

In situ hybridization was performed as described previously (Das *et al.* 1994). Uteri from pregnant mice were cut into 4–6 mm pieces and flash frozen in Histo-Freeze (Fisher Scientific, Pittsburgh, PA, USA). Frozen sections

Table 1 Primers used in qRT-PCR experiments and subcloning for *in situ* hybridization.

Gene	Up primer sequence	Down primer sequence
GAPDH	TGCCCCCATGTTTGTGATG	TGTGGTCATGAGCCCTTCC
18S	CGCCGCTAGAGGTGAAATTCT	CGAACCTCCGACTTTCGTTCT
ERM	AAGAGGTTGCTCGCCGTTG	CCTTCTGCATGATGCCCTTTT
ER81	TGAAAATGGAGCCCCCAATCA	GCTGGTGTTCCTGAAGGCTTC
PEA3	ATCATGCAGAAGGTGGCTGG	CCGGTCAAACCTCAGCCTTCA
ERM subclone (223 bp)	CAGTCAGGAGTTCCTGGAGATAGT	GCACCTCTGAGTCAGCACAGTAAT
ER81 subclone (418 bp)	TTTGCTTAGGCTTGAATGG	CTCAGTTGGAGCCAGAGTCC
PEA3 subclone (531 bp)	CACAGTCCCTTTGTCCCACT	GCCAGGCAGAACTTTGTCTC

(12 µm) were mounted onto poly-L-lysine-coated slides (Polysciences, Inc., Warrington, PA, USA) and fixed in cold 4% paraformaldehyde in PBS. The sections were prehybridized and hybridized at 45 °C for 4 h in 50% formamide hybridization buffer containing the ³⁵S-labeled antisense cRNA probes for ERM, ER81, and PEA3. After hybridization and washing, the sections were incubated with RNase A (20 µg/ml) at 37 °C for 20 min. RNase A-resistant hybrids were detected by autoradiography using Kodak NTB-2 liquid emulsion (Eastman Kodak Co., Rochester, NY, USA). Sections hybridized with the corresponding antisense probe mixed with a tenfold excess of cold antisense RNA served as negative controls. Slides were post-stained with hematoxylin and eosin. *In situ* hybridization experiments were repeated at least three times using independent samples.

Results

qRT-PCR of the PEA3 family of transcription factors in the mouse uterus

We set out to determine the steady-state levels of ERM, ER81, and PEA3 in the peri-implantation mouse uterus by qRT-PCR. Gene specific primers were designed as shown in Table 1. Values were normalized to the relative amounts of GAPDH cDNA. The results showed that all three genes showed higher mRNA expression on days 4 and 5 of pregnancy than other samples (Fig. 1). There was no significant difference between RNA samples from day 5 implantation sites and inter-implantation sites.

ERM was expressed in the mouse ovary and the peri-implantation uterus

The objective of this experiment was to determine cell type-specific expression of ERM in the peri-implantation (days 1, 4, 5, and 8; day 1 = vaginal plug) mouse uterus. As shown in Fig. 2, ERM was expressed at low levels in the uterus on day 1 of pregnancy. On day 4, heightened expression of ERM was noted both in the epithelium and stroma, suggesting that increasing levels of progesterone is an inducing factor. This was consistent with qRT-PCR results in Fig. 1. During the early phase of implantation on day 5, ERM expression was sustained in the stroma, and endometrial glands showed high expression of ERM. Notably, mesometrial stromal cells exhibited intense ERM

expression. On day 8, endometrial expression of ERM decreased, while the developing embryo showed strong expression of ERM mRNA. In cycling mice, ERM mRNA was highly expressed in the ovary with concentrated accumulation in the corpora lutea (Fig. 2). This result suggests a potential role for ERM in ovarian function.

ER81 was expressed in the vasculature in the maternal decidua

Expression of ER81 on day 1 of pregnancy was very low to undetectable (Fig. 3). On days 4 and 5 of pregnancy, very low levels of ER81 expression were noted in the uterine stroma, while epithelial cells were negative. On day 8, the accumulation of ER81 mRNA was predominantly evident in the developing vasculature of the mesometrial bed. This result suggests that ER81 is a marker of endometrial angiogenesis. Thus, ER81 may be implicated in subsequent placental development. ER81 expression in the ovary was undetectable by *in situ* hybridization (data not shown).

PEA3 was expressed in the developing embryo

PEA3 mRNA was undetectable on day 1, but was induced at low levels in the stromal cells on days 4 and 5 of pregnancy (Fig. 4). PEA3 expression on day 5 was especially concentrated around the implanting embryo. It was also noted in the blastocyst. On day 8, basal levels of PEA3 expression were observed in the maternal decidua, while it was highly expressed in the embryo proper. ER81 expression in the ovary was undetectable by *in situ* hybridization (data not shown). This result shows that PEA3 is an embryonic factor from the very early stage of development.

Overall, *in situ* hybridization showed that three members of PEA3 subfamily of transcription factors exhibit distinct cell type-specific expression in the ovary and peri-implantation uterus and embryo during early pregnancy.

Discussion

The highlight of the present investigation was that three ETS transcription factors belonging to the PEA3 subfamily exhibited differential cellular localization in the adult mouse ovary and uterus, and the developing embryo

Figure 1 qRT-PCR analysis of ERM, ER81, and PEA3 in the peri-implantation mouse uterus. Total RNA samples obtained from pregnant mouse uteri were subjected to RT ($n = 4$ or 5 for each group). qRT-PCR was performed by monitoring in real time the increase in fluorescence of the SYBR Green dye as described, using the ABI Prism 7000 Sequence Detection System. For comparison of transcript levels between samples, a standard curve of cycle thresholds for several serial dilutions of a cDNA sample was established and then used to calculate the relative abundance of each gene. Values were then normalized to the relative amounts of GAPDH cDNA, which were obtained from a similar standard curve. All PCR reactions were performed in duplicate. Error bars represent s.d. values. D, day of pregnancy; D5IS, day 5 implantation sites; D5N, day 5 inter-implantation sites; D8M, day 8 myometrium; D8DE, day 8 embryo plus deciduum.

during early pregnancy. Our expression analyses showed that ERM was expressed in the epithelium and stroma on days 4 and 5 of pregnancy, suggesting that this factor is implicated in the early events of uterine preparation and implantation. The expression of ERM in the corpora lutea suggests its role in ovarian functions. The distinct expression of ER81 in the mesometrial bed on day 8 of pregnancy suggested that it is involved in the establishment of the maternal vasculature for subsequent placental development. PEA3 is apparently an embryonic factor for early embryogenesis. A previous report showed that ERM, ER81, and PEA3 are expressed in many developing organs of different cellular origins during mouse embryonic development (Chotteau-Lelievre *et al.* 2001). Our results showed two points of overlapping expression: ERM and PEA3 were expressed in the stroma on day 5 and in the embryo proper on day 8 of pregnancy. Expression did not overlap on other days during early pregnancy, suggesting differential roles for these factors in female reproductive functions.

Except for PEA3 in male fertility (Laing *et al.* 2000), no other member of the ETS transcription factor is implicated directly in the reproductive processes. Previous studies revealed that ERM, ER81, and PEA3 are differentially regulated during mouse embryogenesis, possibly to provide diverse mechanisms of cellular control (Chotteau-Lelievre *et al.* 2001). ETS-1, another member of the ETS family of transcription factors, has been shown to be expressed in the human endometrium during the menstrual cycle and is implicated in uterine angiogenesis (Fujimoto *et al.* 2003). ETS-1, along with ETS-2, also exhibit differential expression patterns during early embryogenesis and organogenesis (Maroulakou *et al.* 1994). Our result supplements these reports that members of the PEA3 subfamily function differentially in early developmental and reproductive events.

ERM, ER81, and PEA3 are highly homologous factors and are known as transcriptional activators. Regulation of these factors at the gene expression level is important for their availability in controlling target genes, while their activity may also be regulated by post-translational modification. Some of the known target genes of members of the PEA3 subfamily are follicle-stimulating hormone receptor, COX-2, stromelysin, osteopontin, matrilysin, and urokinase plasminogen activator (Rorth *et al.* 1990, Crawford *et al.* 2001, Howe *et al.* 2001, Levallet *et al.* 2001, El-Tanani *et al.* 2004). ERM, ER81, and PEA3 are highly conserved in their protein sequences and are thus capable of inducing significant activation of target genes via PEA3 consensus on promoters (Howe *et al.* 2001). It is thus plausible that the PEA3 subfamily of ETS factors function as transcriptional activators of the above-mentioned signaling molecules in reproductive events. Indeed, COX-2 is a critical factor for both ovulation and implantation (Lim *et al.* 1997). Likewise, stromelysin and other proteases are implicated in female reproductive events (Osteen *et al.* 1994,

Figure 2 Cell type-specific expression of ERM in the mouse ovary and peri-implantation uterus. Gene expression in the peri-implantation mouse uterus was assessed by *in situ* hybridization of paraformaldehyde-fixed frozen sections with ^{35}S -labeled cRNA probes. Darkfield photomicrographs of representative uterine sections are shown at $40\times$, except for day 8 uterine sections which are shown at $20\times$. le, luminal epithelium; ge, glandular epithelium; s, stroma; myo, myometrium; bl, blastocyst; CL, corpus luteum; f, follicle.

Schatz *et al.* 1999). It is therefore plausible that genes involved in prostaglandin production and extracellular matrix remodeling are regulated by these transcription factors. We have also described herein that only ERM is detectable in the mouse ovary. Along with previous reports showing that ERM is also expressed in the mouse testis (Hsia & Cornwall 2004), it is possible that ERM is involved in gonadal functions.

Several reports have shown that members of the PEA3 subfamily are dysregulated in mammary tumors (Trimble *et al.* 1993, Shepherd *et al.* 2001). All three members show increased expression in mammary tumors, suggesting their roles in breast cancer progression (Shepherd *et al.* 2001). PEA3 is also associated in tumor progression in ovarian carcinoma (Davidson *et al.* 2003). In several tumor tissues, PEA3 regulates the expression of matrilysin and metallo-

Figure 3 Cell type-specific expression of ER81 in the peri-implantation mouse uterus. Gene expression in the peri-implantation mouse uterus was assessed by *in situ* hybridization of paraformaldehyde-fixed frozen sections with ^{35}S -labeled cRNA probes. Darkfield photomicrographs of representative uterine sections are shown at $40\times$, except for day 8 uterine sections which are shown at $20\times$. le, luminal epithelium; s, stroma; myo, myometrium; bl, blastocyst; em, embryo.

Figure 4 Cell type-specific expression of PEA3 in the peri-implantation mouse uterus. Gene expression in the peri-implantation mouse uterus was assessed by *in situ* hybridization of paraformaldehyde-fixed frozen sections with ^{35}S -labeled cRNA probes. Darkfield photomicrographs of representative uterine sections are shown at $40\times$, except for day 8 uterine sections which are shown at $20\times$. le, luminal epithelium; s, stroma; myo, myometrium; bl, blastocyst; em, embryo.

proteinase-1 (Horiuchi *et al.* 2003, Yamamoto *et al.* 2004), suggesting that this factor is involved in tumor invasion by regulating extracellular matrix remodeling.

Our present study has shown that, in the adult mouse ovary and peri-implantation uterus, ERM is a predominant factor in both tissues. We have preliminary findings that ERM-deficient female mice suffer from infertility with yet to be identified reason(s). While specific role(s) of ERM in female reproduction remains to be investigated, our data have provided information about the possible targets of ERM functions and will help in revealing the mechanism of infertility in ERM-deficient mice.

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References

Arber S, Ladle DR, Lin JH, Frank E & Jessell TM 2000 ETS gene Er81 controls the formation of functional connections between group Ia sensory afferents and motor neurons. *Cell* **101** 485–498.

Brent AE & Tabin CJ 2004 FGF acts directly on the somitic tendon progenitors through the Ets transcription factors Pea3 and Erm to regulate scleraxis expression. *Development* **131** 3885–3896.

Chotteau-Lelievre A, Dolle P, Peronne V, Coutte L, de LY & Desbiens X 2001 Expression patterns of the Ets transcription factors from the PEA3 group during early stages of mouse development. *Mechanisms of Development* **108** 191–195.

Crawford HC, Fingleton B, Gustavson MD, Kurpios N, Wagenaar RA, Hassell JA & Matrisian LM 2001 The PEA3 subfamily of Ets transcription factors synergizes with beta-catenin-LEF-1 to activate matrilysin transcription in intestinal tumors. *Molecular and Cellular Biology* **21** 1370–1383.

Das SK, Wang XN, Paria BC, Damm D, Abraham JA, Klagsbrun M, Andrews GK & Dey SK 1994 Heparin-binding EGF-like growth factor gene is induced in the mouse uterus temporally by the blastocyst solely at the site of its apposition: a possible ligand for interaction with blastocyst EGF-receptor in implantation. *Development* **120** 1071–1083.

Davidson B, Goldberg I, Gotlieb WH, Koplovic J, Ben-Baruch G & Reich R 2003 PEA3 is the second Ets family transcription factor involved in tumor progression in ovarian carcinoma. *Clinical Cancer Research* **9** 1412–1419.

Dey SK 1996 Implantation. In *Reproductive Endocrinology, Surgery and Technology*, pp 421–434. Eds EY Adashi, JA Rock, Z Rosenwaks. New York, NY: Lippincott-Raven.

Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T & Wang H 2004 Molecular cues to implantation. *Endocrine Reviews* **25** 341–373.

El-Tanani M, Platt-Higgins A, Rudland PS & Campbell FC 2004 Ets gene PEA3 cooperates with beta-catenin-Lef-1 and c-Jun in

- regulation of osteopontin transcription. *Journal of Biological Chemistry* **279** 20794–20806.
- Fujimoto J, Aoki I, Toyoki H, Khatun S, Sato E & Tamaya T** 2003 Expression of ETS-1 related to angiogenesis in uterine endometrium during the menstrual cycle. *Journal of Biomedical Science* **10** 320–327.
- Horiuchi S, Yamamoto H, Min Y, Adachi Y, Itoh F & Imai K** 2003 Association of ETS-related transcriptional factor E1AF expression with tumour progression and overexpression of MMP-1 and matrilysin in human colorectal cancer. *Journal of Pathology* **200** 568–576.
- Howe LR, Crawford HC, Subbaramaiah K, Hassell JA, Dannenberg AJ & Brown AM** 2001 PEA3 is up-regulated in response to Wnt1 and activates the expression of cyclooxygenase-2. *Journal of Biological Chemistry* **276** 20108–20115.
- Hsia N & Cornwall GA** 2004 DNA microarray analysis of region-specific gene expression in the mouse epididymis. *Biology of Reproduction* **70** 448–457.
- Laing MA, Coonrod S, Hinton BT, Downie JW, Tozer R, Rudnicki MA & Hassell JA** 2000 Male sexual dysfunction in mice bearing targeted mutant alleles of the PEA3 ets gene. *Molecular and Cellular Biology* **20** 9337–9345.
- Levallet J, Koskimies P, Rahman N & Huhtaniemi I** 2001 The promoter of murine follicle-stimulating hormone receptor: functional characterization and regulation by transcription factor steroidogenic factor 1. *Molecular Endocrinology* **15** 80–92.
- Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM & Dey SK** 1997 Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* **91** 197–208.
- Maroulakou IG, Papas TS & Green JE** 1994 Differential expression of ets-1 and ets-2 proto-oncogenes during murine embryogenesis. *Oncogene* **9** 1551–1565.
- Morrison TB, Weis JJ & Wittwer CT** 1998 Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification. *Biotechniques* **24** 954–958, 960, 962.
- Osteen KG, Rodgers WH, Gaire M, Hargrove JT, Gorstein F & Matrisian LM** 1994 Stromal–epithelial interaction mediates steroidal regulation of metalloproteinase expression in human endometrium. *PNAS* **91** 10129–10133.
- Roehl H & Nusslein-Volhard C** 2001 Zebrafish *pea3* and *erm* are general targets of FGF8 signaling. *Current Biology* **11** 503–507.
- Rorth P, Nerlov C, Blasi F & Johnsen M** 1990 Transcription factor PEA3 participates in the induction of urokinase plasminogen activator transcription in murine keratinocytes stimulated with epidermal growth factor or phorbol-ester. *Nucleic Acids Research* **18** 5009–5017.
- Schatz F, Krikun G, Runic R, Wang EY, Hausknecht V & Lockwood CJ** 1999 Implications of decidualization-associated protease expression in implantation and menstruation. *Seminars in Reproductive Endocrinology* **17** 3–12.
- Sharrocks AD** 2001 The ETS-domain transcription factor family. *Nature Reviews – Molecular Cell Biology* **2** 827–837.
- Shepherd TG, Kockeritz L, Szrajber MR, Muller WJ & Hassell JA** 2001 The *pea3* subfamily ets genes are required for HER2/Neu-mediated mammary oncogenesis. *Current Biology* **11** 1739–1748.
- Trimble MS, Xin JH, Guy CT, Muller WJ & Hassell JA** 1993 PEA3 is overexpressed in mouse metastatic mammary adenocarcinomas. *Oncogene* **8** 3037–3042.
- Wittwer CT, Herrmann MG, Moss AA & Rasmussen RP** 1997 Continuous fluorescence monitoring of rapid cycle DNA amplification. *Biotechniques* **22** 130–138.
- Yamamoto H, Horiuchi S, Adachi Y, Taniguchi H, Nosho K, Min Y & Imai K** 2004 Expression of ets-related transcriptional factor E1AF is associated with tumor progression and over-expression of matrilysin in human gastric cancer. *Carcinogenesis* **25** 325–332.

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