PERGAMON

European Journal of Cancer 38 Supplement 6 (2002) S71–S72

European Journal of Cancer

www.ejconline.com

# Loss of progesterone receptor may lead to an invasive phenotype in human endometrial cancer

E.E. Hanekamp[a,*], E.C.M. Kühne[a], E. Smid-Koopman[b], P.E. de Ruiter[a], S. Chadha-Ajwani[c], A.O. Brinkmann[a], C.W. Burger[b], J.A. Grootegoed[a], F.J.M. Huikeshoven[b], L.J. Blok[a]

[a]*Department of Reproduction & Development, Erasmus MC, Rotterdam, The Netherlands*
[b]*Department of Gynaecology & Obstetrics, Erasmus MC, Rotterdam, The Netherlands*
[c]*Department of Pathology, Erasmus MC, Rotterdam, The Netherlands*

Endometrial cancer is the most common gynaecological malignancy in Northern Europe and the United States. Mortality is low, with surgery as an effective first-line treatment. Endometrial cancer growth is inhibited by progesterone, but when the disease has progressed or when patients have been treated with progesterone for a longer time, the cancer cells lose sensitivity to the hormone.

The progesterone receptor (PR) exists as two isoforms, PRA and PRB. It is known that less differentiated endometrial tumours express less or no PR [1].

We have developed an *in vitro* model for studying the role of the PRs in endometrial cancer, using an epithelial cancer cell line derived from a well differentiated human endometrial adenocarcinoma (Ishikawa). After several passages in culture, this cell line lost expression of PRs and also of oestrogen receptors. We transfected parental Ishikawa cells to create cell lines stably expressing either PRA, PRB, or both receptors. This allows us to study the role of PR isoforms separately, as well as the influence of simultaneous expression of both receptor isoforms on the development of human endometrial cancer.

To study the regulation of gene expression by progesterone in human neoplastic endometrium, Ishikawa cells expressing both PRA and PRB (line PRAB-36), were cultured for 48 h in the absence or presence of medroxyprogesterone acetate (MPA, $10^{-6}$ M), The mRNA from these cells was hybridised to a 9600 cDNA microarray (IncyteGenomics, Palo Alto, CA, USA). Data were analysed, which resulted in the identification of a panel of progesterone-regulated genes. Interestingly, a substantial subgroup consisted of genes involved in tumour cell invasion. For example, CD44 [2] and chondroitin sulphate proteoglycan [3], both involved in cell-matrix interactions, were downregulated by progesterone in our PRAB-36 cell line model. To translate our findings to the clinic, formalin-fixed, paraffin-embedded samples of endometrial cancers, removed at the University Hospital Rotterdam, were immunohistochemically stained to detect PR, CD44, and chondroitin sulphate. We also immunostained sections of these samples to detect E-cadherin. It is widely accepted that expression of this cell-cell adhesion molecule is downregulated in virtually all epithelial tumours expressing a metastatic phenotype [4,5]. E-cadherin was expressed at a high level in the PRAB-36 cell line, but we did not find a direct regulation of E-cadherin by MPA at the level of mRNA. We have analysed the expression of E-cadherin protein in comparison to expression of PR. From these immunostainings, we learned that, as a trend, in tumours where PR expression is lost, CD44 and chondroitin sulphate expression are upregulated, while E-cadherin expression is downregulated. This correlates with a less differentiated histology and more extensive invasion of the tumour into the myometrium.

The downregulation of tumour cell invasion-related genes by MPA in the PRAB-36 cell line seemed to indicate a role for progesterone and the PRs in inhibiting invasion *in vivo*. Loss of PR expression in endometrial cancer could result in the loss of this downregulation of invasion-related genes. This in turn would lead to a relative upregulation of these genes, leading to enhanced invasion. The results of our patient immunohistochemistry stainings seems to confirm this model.

* Corresponding author. Tel.: +31-10-408-7865; fax: +31-10-408-9461.

*E-mail address:* hanekamp@endov.fgg.eur.nl (E.E. Hanekamp).


PII: S0959-8049(02)00294-0

We hypothesise that the loss of PR in human endometrial cancer induces the upregulation of expression of invasion-associated genes and therefore could play a role in the development of a more invasive phenotype. We are currently investigating the *in vitro* and *in vivo* invasive potential of each of the PRA- and/or PRB-expressing Ishikawa cell lines to elucidate whether this is a consequence of specific loss of one of the PR isoforms.

## Acknowledgements

This work was supported by a grant from the Netherlands Organisation for Scientific Research (NWO 903-46-182).

## References

1. Satayaswaroop PG, Mortel R. Sex steroid receptors in endometrial carcinoma (editorial). *Gynecol Oncol* 1993, **50**, 278–280.
2. Smid-Koopman E, Blok LJ, Kuhne ECM, Burger CW, Helmerhost TJM, Brinkmann AO, *et al*. Distinct functional differences of human progesterone receptors A and B on gene expression and growth regulation in two endometrial cancer cells lines. *J Soc Gyn Invest* 2002, submitted.
3. Leblanc M, Poncelet C, Sonano D, *et al*. Alteration of CD44 and cadherins expression: possible association with augmented aggressiveness and invasiveness of endometrial carcinoma. *Virchows Arch* 2001, **438**, 78–85.
4. Iida J, Meijne AML, Knutson JR, Furcht LT, McCarthy JB. Cell surface chondroitin proteoglycans in tumor cell adhesion, motility and invasion. *Semin Cancer Biol* 1996, **7**, 155–162.
5. Beavon IRG. The E-cadherin-catenin complex in tumour metastasis: structure, function and regulation. *Eur J Cancer* 2000, **36**, 1607–1620.