Letters to the Editor

Progestosterone receptor gene polymorphism and risk for breast and ovarian cancer

Sir,

McKenna et al (March, 1995) used Southern analysis to identify a germline TaqI restriction fragment length polymorphism (RFLP) in intron G of the human progesterone receptor (hPR) defined by two alleles, T1 and T2. The T2 allele contained an additional TaqI restriction site relative to T1, and was recently characterized as a 306 bp Alu element insertion and named PROGINS (Rowe et al, 1995). No functional consequences of this intronic insertion have been reported, but McKenna et al (1995) suggested that the T2 allele is over-represented in patients with ovarian carcinoma. Twenty-four out of 67 (36%) German and Irish patients with ovarian cancer were homozygous or heterozygous for the T2 allele, in contrast to only 38 out of 184 (21%) control subjects.

To investigate this association in a Caucasian North-American population, we designed a PCR-based assay using forward (5'-GGC AGA AAG CAA AAT AAA AAG A-3') and reverse (5'-AAA GTA TTT TCT TGC TAA ATG TC-3') primers to amplify the region spanning the insertion. Leucocyte DNA was analysed from 96 patients with ovarian cancer and 68 patients with breast cancer treated at Duke University Medical Center, Durham, NC, USA, between 1985 and 1996, and 101 non-cancer female control subjects enrolled through outpatient clinics at the same hospital. The frequency of T2 genotypes in American control women is similar to that of the pooled Irish/German control subjects. However, we observe no increased frequency in women with the T2 genotype among cases of breast or ovarian cancer relative to controls subjects (Table 1), in contrast to the study by McKenna et al (1995).

In the absence of data that the insertion of an Alu element in intron G of the progesterone receptor gene has any consequences for gene function, we find little support for the hypothesis that the T2 allele increases risk for ovarian or breast cancer.

JM Lancaster,1,2 A Berchuck,3 ME Carney,2 R Wiseman1 and J A Taylor2

1 National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, USA
2 Division of Gynecologic Oncology, Duke University Medical Center, Durham, NC 27710, USA

REFERENCE

McKenna NJ, Kiebuck DG, Carney DN, Fanning M, McLinden J and Headon DR (1995) A germline TaqI restriction fragment length polymorphism in the progesterone receptor gene in ovarian cancer. Br J Cancer 71: 451–455

Rowe SM, Coughlan SJ, McKenna NJ, Garrett E, Kiebuck DG, Carney DN and Headon DR (1995) Ovarian carcinoma-associated TaqI restriction fragment length polymorphism in intron G of the progesterone receptor gene id due to an Alu sequence insertion. Cancer Res 55: 2743–2745

Lymphogenesis and cancer metastasis

Sir,

A recent article by Ohta et al (1997) examines the relationship between lung cancer metastasis and vascular endothelial growth factor (VEGF) expression. The authors suggest that lymph node metastasis may be enhanced by an increase in the number of lymphatic vessels in the primary tumour. They refer to this process as ‘lymphogenesis’. Unfortunately, this term is often used to denote a reference to lymphocyte proliferation or, in some cases, lymph fluid production and, thus, could be misleading in this context. We have suggested the use of the term lymphogenesis to describe the process of lymphatic vessel formation (Cann et al, 1995; van Netten et al, 1996). Another synonymous term, lymphangiogenesis, may be confused with both vascular systems because angiogenesis, first used by Hertig (1935), refers solely to blood vessel formation.

The majority of neovascular research in cancer has focused on angiogenesis. This is partly because of the use of certain techniques, such as corneal implantation or chorioallantoic membrane assays, in which blood vessels can be clearly distinguished and lymphatic vessels, although present, cannot. It is also because of the use of so-called ‘angiogenic’ markers (i.e. von Willebrand factor, CD31, CD34) that are not truly restricted to blood vessel endothelium but that are also present on lymphatic vessels (Miettinen et al, 1994; Appleton et al, 1996).

The process of lymphatic vessel development has gained increasing attention with the discovery of a new VEGF receptor, VEGFR3 (FLT4). VEGFR3 is expressed by lymphatic endothelial cells, some high endothelial venules (Kaipainen et al, 1995) and various tumour cell lines (Pajusola et al, 1992; Liu et al, 1997). Enhanced expression has also been observed in murine hepatic tumours (Karamysheva et al, 1996) and in lymph nodes containing metastatic adenocarcinoma (Kaipainen et al, 1995). The ligand for VEGFR3, VEGF-C, has been shown to be a specific inducer of lymphatic endothelial cell proliferation and chemotaxis (Oh et al, 1997). Thus, lymphogenesis may be a more important factor in the

Table 1 Distribution of hPR polymorphism in control, ovarian, and breast cancer groups

| Group          | T1/T1 (%) | T1/T2 (%) | T2/T2 (%) |
|----------------|-----------|-----------|-----------|
| Control (n = 101) | 79 (78)   | 18 (18)   | 4 (4)     |
| Ovarian cancer (n = 96) | 76 (79)   | 15 (16)   | 5 (5)     |
| Breast cancer (n = 68) | 55 (81)   | 12 (18)   | 1 (1)     |

T1/T1, homozygotes without the 306-bp insertion; T1/T2, heterozygotes with the insertion; T2/T2, homozygotes with the insertion.

British Journal of Cancer (1998) 78(2), 277–278
© 1998 Cancer Research Campaign
spread of some tumours than others. In conclusion, we suggest that a more general term, such as vasogenesis (blood and lymphatic vessel formation), may be more appropriate, unless the two processes of angiogenesis and lymphangiogenesis are clearly distinguished.

**JP van Netten**\(^1\), SA Cann\(^1\), CA Maxwell\(^1\) and RP Finegan\(^1\)

\(^1\)Special Development Laboratory, Royal Jubilee Hospital, Capital Health Region V8R 1J8; \(^2\)Department of Biology, University of Victoria V8W 3N5, Victoria, British Columbia, Canada

**REFERENCES**

Appleton MAC, Attanoos RL and Jasani B (1996) Thrombomodulin as a marker of vascular and lymphatic tumours. *Histopathology* 29: 153–157

Cann SA, van Netten JP, Ashby TL, Ashwood-Smith MJ and van der Westhuizen NG (1995) Role of lymphangiogenesis in neovascularisation. *Lancet* 346: 903

Hertig AT (1935) Angiogenesis in early human chorion and in the primary placenta of the Macaque monkey. *Contrib Embryol* 25: 37–81

Kaipainen A, Korhonen J, Mustonen T, van Hinsberg VW, Fang GH, Dumont D, Breitman M and Altitalo K (1995) Expression of the fms-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development. *Proc Natl Acad Sci USA* 92: 3566–3570

**Lymphangiogenesis and cancer metastasis – reply to the letter from van Netten et al**

Sir,

In their letter, van Netten et al made a point about the clear definition of the term for the neovascularization of lymphatic vessels. They purported ‘lymphangiogenesis’ instead of ‘lymphogenesis’ or ‘lymphangiogenesis’ to describe the process of lymphatic vessel formation. Obviously, uniformity of the term is needed. However, the existence of the neovascularization of lymphatics in tumours is not as yet well documented. In addition, the process of the formation of a lymphatics-rich situation seems to be complicated. As we mentioned in the Discussion, other functions that are different from proliferation of the endothelial cells should be taken into consideration for the relationship between vascular endothelial growth factor (VEGF) and lymph node metastasis. For example, it has been found that angiogenesis has a fundamental role in tumour invasion (Skobe et al, 1997). If the neoformation of lymphatics develops from pre-existing lymphatic vessels as blood vessels do, the existence of pre-existing lymphatic endothelium might be important for the formation of new lymphatics, and tumours with high VEGF expression might be involved in the lymphatics-rich situation in the course of spreading into surrounding tissue and catching lymphatics. Recently, some reports have suggested that the specific pathways involved in the formation of lymphatics are different from those in haemangiogenesis (Wilting et al, 1996; Oh et al, 1997). And among the VEGF family members, the function of VEGF-C appears to extend to the lymphatic system as a ligand for flt-4 (Kukk et al, 1996; Jeltsch et al, 1997, Oh et al, 1997). Although our results suggest that the expression of VEGF has a tendency to be greater in node-positive than in node-negative lung cancer patients, the connection with the number of lymphatics is not yet clear. In human primary cutaneous melanoma, de Waal et al (1997) have recently reported the lack of ‘lymphangiogenesis’, even in the presence of extensive haemangiogenesis, based on the results of an immunohistochemical double-staining method. In cutaneous melanoma, a positive relationship between VEGF expression and lymph node metastasis has also been reported (Sävlen et al, 1997). These results suggest that tumour cells with high VEGF expression may elicit some function for nodal metastasis independent of the number of lymphatics. We believe the potential role of VEGF in lymphatic metastasis warrants further study in this light. In conclusion, it is important that the terminology is clarified; and the answer to the mechanisms of lymphatic metastasis, including the neoformation of lymphatic vessels, will provide the final definition of the term.

Y Ohta

First Department of Surgery, Kanazawa University School of Medicine, Takara-machi 13-1, Kanazawa 920, Japan

**REFERENCES**

de Waal RMW, van Altena MC, Erhard H, Weidhe UH, Nooijen PTGA and Ruiter DJ (1997) Lack of lymphangiogenesis in human primary cutaneous melanoma. *Am J Pathol* 150: 1951–1957

Jeltsch M, Kaipainen A, Joukov V, Meng X, Lakso M, Rauvala H, Swartz M, Fukumura D, Jain RK and Altitalo K (1997) Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. *Science* 276: 1423–1425

Kukk E, Lymboussaki A, Taira S, Kaipainen A, Jeltsch M, Joukov V and Altitalo K. VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development. *Development* 122: 3829–3837

Oh SJ, Jeltsch MM, Birkenhager R, McCarthy JE, Weich HA, Christ B, Altitalo K and Wilting J (1997) VEGF and VEGF-C: specific induction of angiogenesis and lymphangiogenesis in the differentiated avian chorioallantoic membrane. *Dev Biol* 188: 96–109

Sävlen P, Heikillä P and Joneus H (1997) Enhanced expression of vascular endothelial growth factor in metastatic melanoma. *Br J Cancer* 76: 930–934

Skobe M, Rockwell P, Goldstein N, Nosseler S and Fusinger NE (1997) Halting angiogenesis suppresses carcinoma cell invasion. *Nature Med* 3: 1222–1227

Wilting J, Birkenhager R, Eichmann A, Kurz H, Martin-Byron G, Marme D, McCarthy JE, Christ B and Weich HA (1996) VEGF121 induces proliferation of vascular endothelial cells and expression of flk-1 without affecting lymphatic vessels of chorioallantoic membrane. *Dev Biol* 176: 76–85

**British Journal of Cancer (1998) 78(2), 277–278 © Cancer Research Campaign 1998**