Review

Role of genetic polymorphisms in tumour angiogenesis

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Angiogenesis plays a crucial role in the development, growth and spread of solid tumours. Pro- and anti-angiogenic factors are abnormally expressed in tumours, influencing tumour angiogenesis, growth and progression. Polymorphisms in genes encoding angiogenic factors or their receptors may alter protein expression and/or activity. This article reviews the literature to determine the possible role of angiogenesis-related polymorphisms in cancer. Further research studies in this potentially crucial area of tumour biology are proposed.

Keywords: tumour angiogenesis; genetic polymorphism(s)

TUMOUR ANGIOGENESIS

Angiogenesis is a complex cascade of events involving extensive interplay between cells, soluble factors and extra-cellular matrix components. Soluble factors including cytokines have a stimulatory or inhibitory role, thereby regulating the process. The angiogenic potential of tumours was initially demonstrated in animal models and it is now recognised that angiogenesis not only precedes tumour growth, but is also necessary for metastasis. In the normal adult vasculature, a balance of the positive and negative angiogenic signals maintains quiescence. However, in the tumour microenvironment, angiogenesis occurs as there is either a preponderance of pro-angiogenic molecules or a decrease in anti-angiogenic stimuli.

GENETIC POLYMORPHISMS IN ANGIOGENIC GENES AND RELEVANCE TO CANCER CARE

Polymorphisms are naturally occurring DNA sequence variations, which differ from gene mutations in that they occur in the ‘normal’ healthy population and have a frequency of at least 1%. Approximately 90% of DNA polymorphisms are single nucleotide polymorphisms (SNPs) due to single base substitutions. Others include insertion/deletion polymorphisms, minisatellite and micro-satellite polymorphisms. Although most polymorphisms are functionally neutral, some have effects on regulation of gene expression or on the function of the coded protein. These functional polymorphisms, despite being of low penetrance, could contribute to the differences between individuals in susceptibility to and severity of disease. Certain polymorphisms alone, in combination or by interaction with environmental factors may affect the angiogenic pathway and thereby susceptibility and/or severity of cancers. Detection of the role of angiogenic gene polymorphisms that influence cancer susceptibility and/or severity may improve our understanding of tumour angiogenesis and may influence risk stratification and detection, use of new treatment strategies and prognostication of disease. The efficacy of anti-angiogenic treatment in solid cancer (Jain, 2001) could be further enhanced, if the individual angiogenic potential could be predicted on the basis of genotype.

The article reviews the role of polymorphisms in genes encoding factors and receptors that influence tumour angiogenesis. Whilst numerous polymorphisms have been identified, we have confined this review to those that are thought to be functionally important and may influence angiogenesis. Table 1 summarises the population studies that have evaluated a number of the genetic polymorphisms that will be discussed. Some ‘mutations’ with potential functional significance have been discussed briefly, as their prevalence in the normal population is as yet unknown. Factors/genes, which demonstrate minimal or indirect effects on angiogenesis such as tumour suppressor genes, oncogenes, hormones and hematopoietic factors, are not discussed in this review.

VASCULAR ENDOTHELIAL GROWTH FACTOR

Vascular endothelial growth factor (VEGF or VEGF-A) is one of a family of six protein isoforms expressed in different tissues including brain, kidney, liver and spleen. VEGF stimulates proliferation, migration and tube formation of endothelial cells in vitro and regulates vascular permeability in vivo (Veikkola and Alitalo, 1999). VEGF expression correlates with angiogenesis and prognosis in several tumours including breast, lung and malignant mesothelioma (Toi et al, 2001; Strizzi et al, 2001).

Several polymorphisms have been described within the promoter and 5’UTR of the VEGF gene, some of which (+405 C>G, −1154G>A and −2578C>A) correlate with VEGF production. The +405C, −1154G and −2578C alleles are associated with low VEGF production (Watson et al, 2000; Shahhazi et al, 2002a). Recent studies have shown that in individuals with the −1154 AA genotype, there may be a decrease in prostate cancer risk (McCarron et al, 2002) and reduction in invasive potential of malignant melanomas (Howell et al, 2002). This confirms earlier studies that associate reduced VEGF production with the −1154A allele.

In another study of healthy individuals, three polymorphisms (702 C>T, 936 C>T and 1612 G>A) were identified in the 3’UTR region of the VEGF gene. The 936 T allele was associated with decreased
| Gene     | Polymorphism | Rare allele frequency in controls | Cancer                  | Tumour susceptibility | Tumour severity/survival | Number of cases | Number of controls | Reference                      |
|----------|--------------|----------------------------------|-------------------------|-----------------------|--------------------------|------------------|------------------|-------------------------------|
| VEGF    | −1154G>A in promoter | A allele=0.34 | Prostate cancer | AA genotype reduces risk (OR=0.45; 95% CI=0.24–0.86) | No association shown | 238 | 263 | (McCarron et al, 2002) |
|         | −1154G>A in promoter | A allele=0.34 | Malignant melanoma | No association shown | VEGF − 1154AA genotype associated with thinner tumours (P=0.002) | 152 | 266 | (Howell et al, 2002) |
|         | −2578 G>A in promoter +405G>C in 5'UTR | C allele=0.31 | Malignant melanoma | No association shown | No association shown | 134 | 266 | (Howell et al, 2002) |
|         | 936 C>T in 3'UTR | T allele=0.12 | Malignant melanoma | No association shown | No association shown | 144 | 238 | (Howell et al, 2002) |
|         | 936 C>T in 3'UTR | T allele=0.13 | Breast cancer | No association shown | No association shown | 862 | 713 | (Balasubramanian et al, 2002) |
| FGFR4   | Glycine to Arginine change at position 388 | Avg<sup>388</sup> allele=0.31 | Breast cancer | No association shown | Reduced disease free survival for Avg<sup>388</sup> carriers in patients with lymph node metastases (P=0.01) | 84 | 123 | (Bange et al, 2002) |
| EGF     | 61G>A in 5'UTR | G allele=0.44 | Malignant melanoma | G/G genotype associated with increased risk (OR=4.9; 95% CI=2.3–10.2) | G/G genotype associated with increased thickness (P=0.045) | 135 | 99 | (Shahbazi et al, 2002b) |
| Endostatin | G>A in exon 42 (Aspartic acid to Asparagine) | A allele=0.06 | Prostate cancer | Rare allele increases risk (OR=2.4; 95% CI=1.4–4.2) | No association shown | 181 | 198 | (Lughetti et al, 2001) |
|         |               | A allele=0.08 | Breast cancer | No association shown | A allele associated with invasiveness (P=0.046) | 861 | 697 | Unpublished data |
| MMP-1   | −1607 IG/2G | IG allele=0.39 | Ovarian cancer | Common allele increases risk (OR=2; 95% CI=1–4) | Not studied | 163 | 150 | (Kanamori et al, 1999) |
|         |               | IG allele=0.39 | Endometrial cancer | Common allele increases risk (P=0.019) | No association shown | 100 | 150 | (Nishioka et al, 2000) |
|         |               | IG allele=0.25 | Lung cancer | 2G/2G genotype increases risk (OR=1.76; 95% CI=1.3–2.4) | Not studied | 456 | 451 | (Zhao et al, 2001) |
|         |               | – | Colorectal cancer | 2G/2G genotype increases risk (OR=2.2; 95% CI=1.4–2.4) | 2G/2G genotype increases risk of metastases (P=0.008) | 60 | 164 | (Ghildi et al, 2001) |
|         |               | – | Malignant melanoma | No association shown | 2G allele associated with deeper tumours | 139 | No controls | (Ye et al, 2001) |
| MMP-3   | −1612 5A/6A | SA allele=0.48 | Breast cancer | 5A carriers increase risk (P<0.05) | Not studied | 43 | 164 | (Biondi et al, 2000) |
|         |               | – | Colorectal cancer | No association shown | Not studied | 63 | 164 | (Biondi et al, 2000) |
| PAI-1   | −675 4G/5G | 4G allele=0.42 | Breast cancer | No association shown | No association shown | 100 | 106 | (Biswas & Smolarz, 2000) |
|         |               | – | Colorectal cancer | No association shown | No association shown | 40 | No controls | (Smolarz et al, 2001) |
| TNF-α   | −308 G>A | A allele=0.18 | Breast cancer | Homozygous rare allele genotype increases risk (RR=4.44; P=0.006) | No association shown | 711 | 502 | Unpublished data |
|         |               | A allele=0.02 | Breast cancer | Homozygous rare allele genotype decreases disease-free and overall survival (P<0.001) | No association shown | 243 | 174 | (Mestiri et al, 2001) |
|         |               | A allele=0.04 | Breast cancer | No association shown | No association shown | 169 | 92 | Unpublished data |
|         |               | A allele=0.12 | Colorectal cancer | No association shown | No association shown | 140 | 328 | (Park et al, 1998) |
|         | −238 G>A | A allele=0.07 | Breast cancer | A allele decreases risk to all these cancers (OR=0.25; 95% CI=0.1–0.6) | No association shown | 169 | 92 | (Jang et al, 2001b) |
|         |               | A allele=0.06 | Breast cancer | No association shown | No association shown | 714 | 499 | Unpublished data |

*Continued*
VEGF plasma levels (Renner et al., 2000). We have studied this polymorphism in breast cancer, but were unable to demonstrate any significant association (Balasubramanian et al., 2002).

FIBROBLAST GROWTH FACTOR

The fibroblast growth factor (FGF) family includes 20 polypeptide growth factors sharing a central core of 140 amino acids (Powers et al., 2000). Four receptors (FGFR 1 to 4) belonging to the immunoglobulin superfamily have been identified. In addition to effects on inflammation, repair and tissue regeneration, FGFs also stimulate the proliferation and migration of endothelial cells, important in the process of angiogenesis. FGFs promote tumour cell mitosis and angiogenesis and inhibit apoptosis (Powers et al., 2000). Four receptors (FGFR 1 to 4) belonging to the immunoglobulin superfamily have been identified. In addition to effects on inflammation, repair and tissue regeneration, FGFs also stimulate the proliferation and migration of endothelial cells, important in the process of angiogenesis. FGFs promote tumour cell mitosis and angiogenesis and inhibit apoptosis (Powers et al., 2000).

Table 1 (Continued)

Table 2 Summary of the review

- Angiogenic factors play an important role in tumour development and progression
- Potentially functional gene polymorphisms have been described in many angiogenic genes
- Functional polymorphisms may influence tumour susceptibility and/or severity and may help in assessing risk of disease, determining prognosis and deciding the use of specific treatment regimens
- Both large-scale population studies and functional studies of these polymorphisms are urgently required.

EPIDERMAL GROWTH FACTOR

Epidermal growth factor (EGF) exerts effects on cell proliferation and differentiation by binding to a tyrosine kinase receptor EGF receptor (EGFR). Although EGF can have direct effects on tumour cells, it also promotes angiogenesis, predominantly through a mitogenic effect on endothelial cells (Dunn et al., 2000). EGF, acting through its receptor also stimulates the production of vascular endothelial growth factor by tumour cells (Goldman et al., 1993), contributing further to the angiogenic response.

A polymorphism in the 5' UTR of the EGF gene (61 G>A) appears to predispose to the development of malignant melanoma. The G allele is associated with higher in vitro EGF production (Shahbazi et al., 2002b).

HEPATOCYTE GROWTH FACTOR/SCATTER FACTOR

Hepatocyte growth factor (scatter factor; HGF/SF), a heparin-binding glycoprotein binds to a tyrosine kinase receptor, which is the protein product of the c-met proto-oncogene. HGF-Met signalling is involved in developmental and homeostatic processes and also regulates neoplastic growth and progression (To and Tsao, 1998). It stimulates endothelial cell proliferation and migration and regulates vascular endothelial cell growth factor expression in vascular smooth muscle cells (Van Belle et al., 1998). Increased Met and HGF/SF expression occurs in many tumours (To and Tsao, 1998) and the HGF/Met autocrine signal-
ling pathway possibly has an oncogenic role (Takayama et al., 1997).

Somatic mutations of the c-met gene in patients with renal cell carcinoma have been shown to be oncogenic (Jeffers et al., 1997). Met is highly expressed in human gastric carcinoma cell lines, and a mutation (germ line missense Met mutation, P1009S) recently identified in gastric cancer displays increased and persistent tyrosine phosphorylation, when treated with HGF. Activating missense Met mutations could therefore contribute to gastric cancer tumorigenesis (Lee et al., 2000). Further investigations are required to determine the role of polymorphisms in the Met gene in angiogenesis and tumorigenesis.

TRANSFORMING GROWTH FACTOR-BETA

Transforming growth factor-beta (TGF-β) is a 25-kDa protein which binds to three membrane molecules, TGF-β receptors type I, II and III (Koli and Keski-Oja, 1996). TGF-βs are potent regulators of cellular proliferation, differentiation and morphogenesis, as well as extra-cellular matrix formation, extra-cellular proteolysis, and inflammation. Although TGF-β inhibits cell proliferation, neoplastic cells acquire resistance to this inhibitory activity. TGF-β induces angiogenesis (Pertovaara et al, 1994) and this, together with effects on stromal formation and immune function, suggests involvement in tumour progression (Gregoire and Lieubeau, 1995). In non-small cell lung cancer, TGF-β1 protein level correlates with microvessel density and prognosis (Hasegawa et al, 2001).

Several polymorphisms have been identified in the TGF-β1 gene and have been studied in ischaemic heart disease (Andreotti et al, 2002). One coding polymorphism, (T>C resulting in Leucine to Proline change) has been associated with serum TGF-β1 levels; individuals with CC genotype having higher levels than TT or TC genotype (Yokota et al, 2000). Mutations have also been identified in the exons of TGF-β1 gene in breast tumour samples (Cardillo et al, 1997a), ovarian tumours (Cardillo et al, 1997b) and colorectal carcinomas (Cardillo and Yap, 1997). However, as yet no correlation has been identified between these mutations and mRNA and/or protein expression in tumours.

TGFBR2, a receptor for TGF-β lies close to or within one of the interstitial deletions that occur in 30–50% of head and neck, breast, and small cell lung cancers (Lucke et al, 2001). TGFBR2 gene mutations occur in colorectal and breast cancers and can result in absent receptor expression at the cell surface and defective TGF-β signalling pathways (Lucke et al, 2001). Potentially, these mutations may have a significant influence on tumour progression.

ENDOSTATIN

Endostatin is a cleavage product of the COOH-terminal domain of collagen XVIII, which inhibits endothelial cell proliferation in vitro and tumour angiogenesis and growth in vivo (O’Reilly et al, 1997). Endostatin inhibits the growth of melanoma, fibrosarcoma, renal cell, mammary and ovarian cancer (Sim et al, 2000), and is currently being assessed in clinical trials.

Several polymorphisms have been described in the Endostatin gene (chromosome 21) (Lughetti et al, 2001). One polymorphism, (G>A in exon 42), which results in the change of aspartic acid to asparagine, is associated with prostate cancer susceptibility (Lughetti et al, 2001). In our study of breast cancer patients, the rare allele of this polymorphism appears to predispose to tumour invasion (unpublished data).

MATRIX METALLOPROTEINASES

The matrix metalloproteinases (MMPs) are a family of highly conserved zinc-dependent endopeptidases with 20 members identi-
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by both normal and tumour cells. It plays a major role in embryogenesis, ovulation, wound healing, inflammation, rheumatoid arthritis and in cancer growth, angiogenesis and metastasis (Hildbrandt et al, 1995; Rabhani, 1998). High levels of uPA, uPA receptor (uPAR) and paradoxically PAI-1 are associated with a poor prognosis in many human cancers (Nekarda et al, 1994; Pedersen et al, 1994; Foekens et al, 2000). This may be due to excess PAI-1 release facilitating re-implantation of circulating tumour cells, as stroma formation at the metastatic site requires the blockade of uPA-mediated degradation of the extracellular matrix (Janicke et al, 1991).

Two polymorphisms have been identified in the uPA gene (Conne et al, 1997). A polymorphic microsatellite marker exists in the uPAR gene, certain alleles of which have been found in colorectal cancer cell lines but not in healthy individuals (Kohonen-Corish et al, 1996). The PAI-1 gene has several polymorphic loci including a 3’ HindIII restriction fragment length polymorphism, a CA(n) dinucleotide repeat in intron 3 and a 4G/5G insertion/deletion at position –675 in the promoter. The smaller dinucleotide repeats are associated with higher plasma PAI-1 levels (Dawson et al, 1991) and the –675 4G allele demonstrates increased PAI-1 activity when compared to the 5G allele (Panahloo et al, 1995). Studies of the 4G/5G polymorphism in small numbers of breast (Blaiki and Smolarz, 2000) and colorectal cancer patients (Smolarz et al, 2001) have not revealed any association with cancer. Larger studies are required to establish a role for polymorphisms in the PAI-1 gene.

CYTOKINES

In addition to specific angiogenic factors, certain cytokines are also involved in regulation of angiogenesis. Cytokine gene polymorphisms with a specific role in angiogenesis are therefore reviewed in the following sections.

Tumour necrosis factor

TNF-α Tumour necrosis factor (TNF-α) plays a critical role in the pathogenesis of various inflammatory, autoimmune and malignant diseases (Bazzoni and Beutler, 1996). Initially thought to have anti-tumour effects, TNF-α was later shown to be tumorigenic in vivo, with high plasma TNF-α levels associated with poor disease outcome (Warzocha et al, 1997). Stimulation of angiogenesis by TNF-α is well recognised, with effects modulated by other angiogenic factors (Yoshida et al, 1997).

Functional polymorphisms in the promoter region of the TNF-α gene at position –308 and –238 are associated with increased severity of infectious diseases, autoimmune diseases, and non-Hodgkin’s lymphomas (Tsukasaki et al, 2001). The functional significance of several other TNF-α polymorphisms have recently been reviewed in detail (Hajeer and Hutchinson, 2001). The uncommon allele of the –308 polymorphism (TNF2) is associated with higher constitutive and inducible levels of TNF-α (Wilson et al, 1997). Individuals with the TNF2 homozygous genotype demonstrate an increased predisposition to breast cancer (RR, 4.44; P=0.006). In addition, the TNF2 homozygous genotype appears to be an independent prognostic indicator for both disease free survival and overall survival (Meisiri et al, 2001). However, this polymorphism does not influence colorectal cancer risk or severity (Park et al, 1998). The –238 A allele has been reported to be protective against cancers in general (Jang et al, 2001b), but this needs to be confirmed in larger studies. We have recently studied both the –308 and –238 polymorphisms in 711 breast cancer patients and 498 age and sex-matched controls, but were unable to demonstrate any association (unpublished data). Three additional polymorphisms located in the 5’-flanking promoter/enhancer region of the TNF-α gene at positions –1031 (T>C), –863 (C>A), and –857 (C>T) are associated with TNF-α production, all rare alleles being associated with higher levels (Higuchi et al, 1998). The TNF-α–857T allele is associated with adult T-cell leukaemia/lymphoma in the Japanese population (Tsukasaki et al, 2001). The effects on angiogenesis and the presence of many functional polymorphisms in the TNF-α gene certainly warrant further study in neoplastic diseases.

TNF-β (Lymphotixin-α) Structurally related to TNF-α, stimulates the production of VEGF in prostate cancer cell lines (Ferrer et al, 1998). Increased serum levels have been associated with progression in cervical cancer (Chopra et al, 1998). A Nco I RFLP exists in the first intron of TNFB (gene for TNF-β); the rare allele of which (TNFB*2) is associated with reduced TNF-β production (Messer et al, 1991). The homozygous common allele genotype (TNFB*1/ TNFB*1) seems to protect against lung cancer (Shimura et al, 1994), colorectal cancer (Park et al, 1998) and breast cancer (Park et al, 2002). Studies on gastric cancer have also shown a prolonged survival in patients with this genotype (Shimura et al, 1995), whereas no association with either susceptibility or survival was demonstrated in pancreatic cancer (Barber et al, 1999).

Interferons

Interferons (IFN) have a multitude of biological effects on growth and immunity, including modulation of gene expression, inhibition of viral replication, immunomodulation, decreased cell proliferation, suppression of oncogene expression and alterations in differentiation. There are three main types: Interferon-α, Interferon-β (or Type 1 Interferons) and Interferon-γ (Type II interferon). Several mechanisms, including inhibition of tumour angiogenesis, mediate the anti-tumour effects (Lindner and Borden, 1997). Interferon-α inhibits angiogenesis by down-regulating the expression of FGF-2 (Dimney et al, 1998). Interferon-β inhibit angiogenesis, possibly by enhanced IFN-induced gene expression and this effect is enhanced when combined with Tamoxifen (Lindner and Borden, 1997).

Several polymorphisms have been described in the interferon-α genes (Golovleva et al, 1996; Muldoon et al, 2001). However, as majority of the members of the IFN-α family have widely overlapping functions, mutations in any one of the several encoding genes may only result in minor functional consequences.

Interferon-γ induces expression of IFN-inducible protein 10 (IP-10), a potent inhibitor of angiogenesis and tumour growth in vivo (Sgadari et al, 1996).

Of the several polymorphisms described in the IFN-γ gene, a CA repeat polymorphism in the first intron has five alleles; the common allele (allele 2, 24 bp long) correlates significantly with high levels of in vitro IFN-γ production (Pravica et al, 1999). Differential binding of nuclear factors has been reported at a polymorphism (+4766 C>T) in the 3’UTR (Bream et al, 2000). As yet, these have not been associated with pathologies such as cancer.

Interleukins

Some Interleukins such as IL-8, IL-12, IL-10 and IL-4 influence tumour growth and angiogenesis by different mechanisms. Although several polymorphisms have been described in the encoding genes, only a few have been shown to be of functional importance. One promoter IL-10 polymorphism (–1082G>A) influences IL-10 production. The G allele (–1082G) is associated with higher cytokine production (Turner et al, 1997) and may increase cervical cancer risk (Stanczuk et al, 2001) and cutaneous malignant melanoma (Howell et al, 2001). At one of the IL-4 polymorphisms (–590C>T), the –590T allele is associated with increased promotor activity (Rosenwasser et al, 1995). This and other polymorphisms if proven to be of functional significance.
could represent potentially significant candidate genes in the regulation of tumour angiogenesis.

STUDY DESIGN AND STATISTICAL CONSIDERATIONS

Genetic polymorphisms are being increasingly evaluated for their role in multifactorial conditions, including cancer, using population case–control studies. Such studies offer many advantages when compared to family studies including:

- Recruitment of large numbers of cases and controls.
- Detection of polymorphisms that confer relative risks as low as 1.5, which is not usually possible with family studies (Risch, 2000), thus allowing identification of low penetrance susceptibility loci.
- As cancers largely affect the middle and elderly age group, family studies like the transmission disequilibrium test and the affected sib pair analysis involving parents and sibs of patients are difficult to perform, as many will be deceased.

However, choosing an ideal control set for a population study is a difficult problem, as the age, sex and ethnicity of the case and control groups should be matched to enable appropriate conclusions to be made. Studies on functional gene polymorphisms will be more likely to yield positive results than random polymorphisms simply because of the greater prior probability of being associated with disease. However, polymorphisms in coding regions resulting in a non-conservative amino-acid substitution in conserved regions of the genome, or in potential transcription factor binding sites, are also studied because of their potential functionality. Other variants, even if not functional, can be associated with phenotype because of linkage to closely situated functional polymorphisms. It is now recognized that specific combinations of polymorphisms in a gene (haplotypes) might be of greater significance than individual polymorphisms, not only for a more efficient capture and analysis of common genetic variation (Johnson et al, 2001), but also from a functional view point (Daly and Day, 2001).

The numbers of patients and appropriately matched controls, needed to demonstrate a specific relative risk with adequate power and acceptable type I error risk in a case–control study would depend on the frequency of the polymorphism in the population. For example, to study a polymorphism with a rare allele frequency of 10% (expected to be associated with cancer with an odds ratio of 1.5) with a power of 80% and type I error rate of 0.05, 558 individuals would be required in each group. Studying rare polymorphisms (<5% rare allele frequency) requires thousands of patients to prove small associations (odds ratio of 1.5 or lower), which may be of little biological interest because of the rarity of the polymorphism in the general population. A detailed discussion of these and related issues can be found in several recent reviews (Daly and Day, 2001; Risch, 2000; Weinberg and Umbach, 2000).

CONCLUSION

Angiogenesis is a multifactorial process regulated by a plethora of factors. Alteration in protein and/or receptor expression plays an important role in tumour angiogenesis and progression. Polymorphisms in the angiogenic genes/factors may in part explain the variation in tumour angiogenesis observed between individuals. The functional significance of polymorphisms can be determined by both in vivo studies and in vitro studies. Simultaneously, well-designed, large case–control studies are necessary to establish associations between polymorphisms and cancer, but as yet there are few such studies.

Individual polymorphisms, even if proven to be functional, may only contribute to (and not solely determine) the heritable variation in protein levels and/or function. Many protein molecules acting along different carcinogenic pathways influence the development and spread of tumours, and hence the final outcome. It is therefore possible that specific combinations of polymorphisms within one or several genes will have a greater impact on the final phenotype than the individual polymorphisms.

We have recently established a DNA repository containing samples of over 1800 breast cancer patients and controls; primarily to identify gene polymorphisms in angiogenesis-related genes that play an important role in tumour growth and progression. We have investigated SNPs in genes including TNF-α, VEGF and Endostatin for associations with breast cancer severity and susceptibility. Functional SNPs in the TNF-2 promoter (−308G>A and −238G>A), in the 3'UTR of the VEGF gene (956C>T) (Balasubramanian et al, 2002) and in exon 42 of the Endostatin gene (G>A change) are not associated with breast cancer. However, the Endostatin polymorphism appears to predispose to breast tumour invasion (unpublished data).

Identification of the role of angiogenesis related gene polymorphisms in the pathogenesis of specific tumours would lead to an increased understanding of the disease process and potentially to risk stratification and prognostication. At the present time, polymorphisms in the VEGF, MMP and PA system and TNF genes seem to be promising in the quest for markers influencing the severity and extent of tumour angiogenesis. In parallel with the search for functional polymorphisms in angiogenesis related genes, epidemiological studies to detect associations of gene polymorphisms with disease phenotypes are desired.

REFERENCES

Andreotti F, Porto I, Crea F, Maseri A (2002) Inflammatory gene polymorphisms and ischaemic heart disease: review of population association studies. Heart 87: 107 – 112.
Apte SS, Mattei MG, Olsen BR (1994) Cloning of the CDNA encoding human tissue inhibitor of metalloproteinases-3 (TIMP-3) and mapping of the TIMP3 gene to chromosome 22. Genomics 19: 86 – 90.
Balasubramanian SP, Brown NJ, Reed MW (2002) Role of a polymorphism in the 3' untranslated region of vascular endothelial growth factor gene in breast cancer. Br J Surg 89: 1.
Bange J, Prechtl D, Cheburkin Y, Specht K, Harbeck N, Schmitt M, Kayazeva T, Muller S, Gartner S, Sures I, Wang H, Imyanitov E, Haring HU, Knayzev P, Iacobelli S, Holter H, Ullrich A (2002) Cancer progression and tumour cell motility are associated with the FGFRA3 Arg388 allele. Cancer Res 62: 840 – 847.
Barber MD, Powell JJ, Lynch SF, Gough NJ, Fearon KC, Ross JA (1999) Two polymorphisms of the tumour necrosis factor gene do not influence survival in pancreatic cancer. Clin Exp Immunol 117: 425 – 429.
Bazzoni F, Beutler B (1996) The tumor necrosis factor gene polymorphisms of the tumour necrosis factor gene do not influence survival in pancreatic cancer. Clin Exp Immunol 117: 425 – 429.
Beranek M, Kankova K, Muzik J (2000) Identification of novel common polymorphisms in the promoter region of the TIMP-3 gene in Czech population. Mol Cell Probes 14: 265 – 268.
Biordil ML, Turti O, Levi S, Seminati R, Cucchetti F, Bernini M, Gilardini G, Guagnellini E (2000) MMP1 and MMP3 polymorphisms in promoter regions and cancer. Clin Chem 46: 2023 – 2024.
Blasiak J, Smolarz B (2000) Plasminogen activator inhibitor-1 (PAI-1) gene promoter polymorphism is not associated with breast cancer. Acta Biochim Pol 47: 191 – 199.
Borghaei RC, Sullivan C, Mochan E (1999) Identification of a cytokine-induced repressor of interleukin-1 stimulated expression of stromelysin 1 (MMP-3). J Biol Chem 274: 2126 – 2131

Jehring BM, Carrington M, O’Toole S, Dean M, Gerrard B, Shin HD, Kosack D, Modi W, Young HA, Smith MW (2000) Polymorphisms of the human IFNG gene noncoding regions. Immunogenetics 51: 50 – 58

Cardillo MR, Yap E (1997) TGF-beta1 in colonic neoplasia: a genetic molecular and immunohistochemical study. J Exp Clin Cancer Res 16: 281 – 288

Cardillo MR, Yap E, Castagna G (1997a) Molecular genetic analysis of TGF-beta1 in breast cancer. J Exp Clin Cancer Res 16: 57 – 63

Cardillo MR, Yap E, Castagna G (1997b) Molecular genetic analysis of TGF-beta1 in ovarian neoplasia. J Exp Clin Cancer Res 16: 49 – 56

Chopra V, Dinh TV, Hannigian EV (1998) Circulating serum levels of cytokines and angiogenic factors in patients with cervical cancer. Cancer Invest 16: 152 – 159

Compagni A, Wilgenbus P, Impagnatiello MA, Cotten M, Christofori G (2000) Fibroblast growth factor 2 and its receptor FGFR-1 in human pros-tate cancer. Mol Biol Cell 11: 124 – 133

Dinney CP, Bielenberg DR, Perrotte P, Reich R, Eve BY, Bucana CD, Fidler IJ (2000) Growth factors in glioma angiogenesis: advantages and potential pitfalls. Br J Clin Pharmacol 50: 489 – 499

Dowson S, Hamsten A, Henney CS, W捎orek ML, Wei M, W捎orek JS, Borden EC (1991) Genetic variation at the plasminogen activator inhibitor-1 locus is associated with altered levels of plasma plasminogen activator inhibitor-1 activity. Arterioscler Thromb Vesi 11: 183 – 190

Dunne IF, Chee O, Black PM (2000) Growth factors in glioma angiogenesis: FGFRs, PDGFR, EGF, and TGFs. J Neurooncol 60: 25 – 31

Ferrari MA, Villa JL, Andrawis RI, Kurtzman SH, Albertsen PC, Gould KL (1999) Proteolysis in human breast and ovarian neoplasia. J Exp Clin Cancer Res 18: 49 – 56

Ferrer FA, Miller LJ, Andrawis RI, Kurtzman SH, Albertsen PC, Laudone VP, Goldman CK, Kim J, Wong WL, King V, Brock T, Gillespie GY (1993) Variation at the plasminogen activator inhibitor-1 locus is associated with altered levels of plasma plasminogen activator inhibitor-1 activity. Arterioscler Thromb Vesi 11: 183 – 190

Garbett EA, Reed MW, Brown NJ (1999) Proteolysis in human breast and ovarian neoplasia. J Exp Clin Cancer Res 18: 49 – 56

Ghilardi G, Biondi ML, Mangoni J, Leviti S, DeMonti M, Guagnellini E, Scorza R (2001) Matrix metalloproteinase-1 promoter polymorphism 1G/2G is correlated with colorectal cancer invasiveness. Clin Cancer Res 7: 2344 – 2349

Golovleva I, Kanderer-Szerszen M, Beckman L, Lundgren E (1996) Polymorphism in the interferon-alpha gene family. Am J Hum Genet 59: 570 – 578

Gregoire M, Lieubeau B (1995) The role of fibroblasts in tumor behavior. Cancer Metastasis Rev 14: 339 – 350

Hajjar AH, Hutchinson DJ (2001) Influence of TNFalpha gene polymorphisms on TNFalpha production and disease. Hum Immunol 62: 1191 – 1199

Hart KC, Robertson SC, Kanemitsu MY, Meyer AN, Tynan JA, Donovan DJ (2000) Transformation and Stat activation by derivatives of FGFR1, FGFR3, and FGFR4. Oncogene 19: 3309 – 3320

Hasegawa Y, Takahashi K, Kanekura T, Tsuchiya T, Imai T, Okumura K (2001) Transforming growth factor-beta1 level correlates with angiogenesis, tumor progression, and prognosis in patients with nonsmall cell lung carcinoma. Cancer 91: 964 – 971

Higuchi T, Seki N, Kaminizo S, Yamada A, Kimura A, Kato H, Itoh K (1998) Polymorphism of the 5'-flanking region of the human tumor necrosis factor (TNF)-alpha gene in Japanese. Tissue Antigens 51: 605 – 612

Hildrebrand R, Dilger I, Horlin A, Statte HJ (1995) Urokinase and macrophages in tumor angiogenesis. Br J Cancer 72: 818 – 823

Howell WM, Bateman AC, Turner SJ, Collins A, Theaker JM (2002) Influence of vascular endothelial growth factor single nucleotide polymorphisms on tumor development in cutaneous malignant melanoma. Genes Immun 3: 229 – 232

Howell WM, Turner SJ, Bateman AC, Theaker JM (2001) IL-10 promoter polymorphisms influence tumor development in cutaneous malignant melanoma. Genes Immun 2: 25 – 31

Iughetti P, Suzuki O, Godoi PH, Alves VA, Sertie AL, Zorick T, Soares F, Camargo A, Moreira ES, di Loreto C, Moreira-Filho CA, Simpson A, Oliva G, Passos Bueno MR (2001) A polymorphism in endostatin, an angiogenesis inhibitor, predisposes for the development of prostatic adenocarcinoma. Cancer Res 61: 7375 – 7378

Jain RK (2001) Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. Nat Med 7: 987 – 989

Jang JH, Shin KH, Park JG (2001a) Mutations in fibroblast growth factor receptor 2 and fibroblast growth factor receptor 3 genes associated with human gastric and colorectal cancers. Cancer Res 61: 3541 – 3543

Jang WH, Yen YI, Yeh SS, Lee YJ, Chun JH, Kim HY, Kim MS, Paik KH (2001b) The −238 tumor necrosis factor-alpha promoter polymorphism is associated with decreased susceptibility to cancers. Cancer Lett 166: 41 – 46

Janicke F, Schmitt M, Graeff H (1991) Clinical relevance of the urokinase-type- and tissue-type-plasminogen activator and of their type 1 inhibitor in patients with cancer. Semin Oncol 18: 300 – 317

Jeffers M, Schmidt L, Nakaigawa N, Webb CP, Weirich G, Kishida T, Zbar B, Vande Woude GF (1997) Activating mutations for the met tyrosine kinase receptor in human cancer. Proc Natl Acad Sci USA 94: 11445 – 11450

Johnson GC, Espósito L, Barratt BJ, Smith A, Heward J, Di Genova G, Ueda H, Cordell HJ, Eaves JA, Duddridge F, Twells RC, Payne F, Hughes W, Nuttland S, Stevens H, Carr P, Tsuomiläo-Wolf E, Tuomi L, Gough SC, Clayton DG, Todda AA (2001) Haplotype tagging for the identification of common disease genes. Nat Genet 29: 233 – 237

Jormo S, Ye S, Moritz J, Walter DH, Dimmeler S, Zeiher AM, Henney A, Hamsten A, Eriksson P (2000) Allele-specific regulation of matrix metallo-proteinase-12 gene activity is associated with coronary artery luminal dimensions in diabetic patients with manifest coronary artery disease. Circ Res 86: 998 – 1003

Kanamori Y, Matsushita M, Minaguchi T, Kobayashi K, Sagas S, Kudo R, Terakawa N, Nakamura Y (1999) Correlation between expression of the matrix metalloproteinase-1 gene in ovarian cancers and an insertion/deletion polymorphism in its promoter region. Cancer Res 59: 4225 – 4227

Kohonen-Corish MR, Wang Y, Doe WF (1996) A highly polymorphic CA/GT repeat in intron 3 of the human urokinase receptor gene (PLAUR). Hum Genet 97: 124 – 125

Koli K, Keski-Oja J (1996) Transforming growth factor-beta system and its regulation by members of the steroid-thyroid hormone superfamily. Adv Cancer Res 70: 63 – 94

Lee JH, Han SU, Cho H, Jennings B, Gerrard B, Dean M, Schmidt L, Zbar B, Vande Woude GF (2000) A novel germ line juxtamembrane Met mutation in human gastric cancer. Oncogene 19: 4947 – 4953

Lindner DJ, Borden EC (1997) Effects of tamoxifen and interferon-beta or the combination on tumor-induced angiogenesis in its promoter region. Cancer Res 57: 4225 – 4227

McCarron SL, Edwards S, Evans PR, Gibbs R, Dearnley DP, Dowie A, Southgate C, Easton DF, Eeles RA, Howell WM (2002) Influence of cytokine gene polymorphisms on the development of prostate cancer. Cancer Res 62: 3369 – 3372

Messer G, Spangler U, Jung MC, Honold G, Blomer K, Pape GR, Riethmüller G, Weiss EH (1991) Polymorphic structure of the tumor necrosis factor (TNF) locus: An Ncol polymorphism in the first intron of the human TNF-beta gene correlates with a variant amino acid in position 26 and a reduced level of TNF- beta production. J Exp Med 173: 199 – 219

Mestiri S, Bouaouina N, Ahmed SB, Khedhaier A, Jrad BB, Remadi S, Chopra V, Dinh TV, Hannigian EV (1998) Circulating serum levels of cytokines in patients with cervical cancer. Cancer Invest 16: 7375 – 7378

Muldoon J, Uriel A, Khoo S, Ollier WE, Hajjar AH (2001) Novel IFN-alpha receptor promoter polymorphisms. Genes Immun 2: 159 – 160

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Yoshimura N, Sano H, Hashimoto A, Yamada R, Nakajima H, Kondo M, Oka T (1998) The expression and localization of fibroblast growth factor-1 (FGF-1) and FGF receptor-1 (FGFR-1) in human breast cancer. *Clin Immunol Immunopathol* 89: 28–34

Zhang B, Henney A, Eriksson P, Hamsten A, Watkins H, Ye S (1999a) Genetic variation at the matrix metalloproteinase-9 locus on chromosome 20q12.2-13.1. *Hum Genet* 105: 418–423

Zhang B, Ye S, Herrmann SM, Eriksson P, de Maat M, Evans A, Arveiler D, Luc G, Cambien F, Hamsten A, Watkins H, Henney AM (1999b) Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. *Circulation* 99: 1788–1794

Zhu Y, Spitz MR, Lei L, Mills GB, Wu X (2001) A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter enhances lung cancer susceptibility. *Cancer Res* 61: 7825–7829