Short Communication

Glomeruloid microvascular proliferation is associated with p53 expression, germline BRCA1 mutations and an adverse outcome following breast cancer

MATERIALS AND METHODS

A total of 292 consecutive Ashkenazi Jewish women aged 65 years or less with primary nonmetastatic breast cancer diagnosed at one Montreal institution between 1980 and 1995 were assessed. Sufficient follow-up and tissue were available for 251 subjects. Following ethics committee approval, specimens were evaluated by one pathologist (LR Bégin) using conventional methods. Accumulation of p53 protein was detected by immunohistochemistry as previously described (Yuan et al, 1999). Pathology blocks from all women were tested for founder BRCA1 mutations (185delAG, n = 18; 5382insC, n = 10) and BRCA2 mutation (6174delT, n = 8) that are common in this population, using established techniques (Foulkes et al, 1997).

Staining of endothelial cells by Factor-VIII (A-0082, Dako, Copenhagen) was performed on formalin-fixed and paraffin-
RESULTS

In all, 43 breast cancers (17%) had one or more GMP, with 36 tumours in group 1 and seven in group 2. Their presence was associated with higher nuclear grade (P for trend <0.0001), oestrogen receptor (ER) negativity (OR 4.7, 95% CI: 2.3–9.6), p53 immunohistochemical positivity (OR 4.1, 95% CI: 2.0–8.2), and germline BRCA1 mutations (odds ratio (OR) 2.6, 95% CI: 1.1–6.3), but not tumour size, axillary nodal status, microvascular density (MVD) or germline BRCA2 mutations (Table 1). There was no relationship between higher GMP grouping and higher MVD or BRCA1 mutation type.

There were 65 breast cancer deaths in this series of women at 10 years follow-up. Kaplan–Meier survival analysis showed that 50.3% of women with GMP died of breast cancer over this period, whereas the mortality was 25.7% for those with no identified GMP (P = 0.0003). Microvascular density was not significantly associated with a worse prognosis (P = 0.47). In a Cox proportional hazards model, the presence of GMP (defined continuously) was associated with a worse prognosis (P = 0.0001). Microvascular density was not significantly associated with a worse prognosis (P = 0.0001). Microvascular density was not significantly associated with a worse prognosis (P = 0.0001). Microvascular density was not significantly associated with a worse prognosis (P = 0.0001). Microvascular density was not significantly associated with a worse prognosis (P = 0.0001). Microvascular density was not significantly associated with a worse prognosis (P = 0.0001). Microvascular density was not significantly associated with a worse prognosis (P = 0.0001). Microvascular density was not significantly associated with a worse prognosis (P = 0.0001).

DISCUSSION

This study is the first to demonstrate that GMP is associated with p53 expression and the presence of germline BRCA1 mutations and it suggests that the presence of GMP is an independent risk factor for death from breast cancer comparable in magnitude to conventional prognostic factors (RR 1.9).

Notably, GMP was not associated with a higher MVD, and the latter was not prognostic for poor survival in our cohort of patients. Vascular endothelial growth factor is implicated in the

**Table 1** Patient characteristics

| Characteristic* | Subjects with GMP (percent) n = 43 | Subjects without GMP (percent) n = 208 | P-value |
|-----------------|-----------------------------------|--------------------------------------|---------|
| Age, median (range) | 50.1 (31.6–65.9) | 53.2 (26.5–65.3) | 0.57 |
| Tumour size, median (cm) | 2.0 | 1.8 | 0.15 |
| Nuclear grade (249) | | | |
| 1 | 2 (4) | 61 (30) | <0.0001* |
| 2 | 15 (35) | 84 (41) | |
| 3 | 26 (60) | 61 (30) | |
| Oestrogen receptor (247) | | | |
| Positive | 14 (33) | 144 (70) | <0.0001 |
| Negative | 28 (67) | 61 (30) | |
| Lymph node status (228) | | | |
| Positive | 17 (44) | 90 (48) | 0.73 |
| Negative | 22 (56) | 99 (52) | |
| Microvascular density (251) | | | |
| Median (range) | 112.5 (43.8–306.3) | 115.6 (37.5–393.8) | 0.27 |
| BRCA-carrier status | | | |
| BRCA1 carrier | 9 (21) | 19 (9) | 0.04 |
| BRCA2 carrier | 1 (2) | 7 (3) | 0.99 |
| Non-carrier | 33 (77) | 182 (88) | |
| p53 IHC (245) | | | |
| Positive | 21 (50) | 40 (20) | |
| Negative | 21 (50) | 163 (80) | 0.0001 |

GMP = glomeruloid vascular proliferation; IHC = immunohistochemistry.

*Number in parenthesis indicates cases with available data. *For trend.
brane (GMP) is a highly characteristic lesion resulting from a gene 
expression profile that is as yet undefined. As antiangiogenic 
therapy is currently under intense investigation, it will be 
required to analyse these therapies.

Angiogenesis is a complex process and its full understanding 
will require analysis at the level of morphology and gene 
expression. Here, we describe the poor prognosis associated 
with GMP, which is a highly characteristic lesion resulting from 
a gene expression profile that is as yet undefined. As antiangiogenic 
therapy is currently under intense investigation, it will be 
important to establish whether the presence of GMP alters the 
effectiveness of such therapies.

ACKNOWLEDGEMENTS

We thank Ann-Josée Paradis for assistance with mutation 
identification. Grants to WDF from the Canadian Genetic Diseases 
Network, the Fonds de la Recherche en Santé du Québec (FRSQ) 
Cancer Network-Breast and Ovarian Tumour Bank Axis and the 
Norwegian Cancer Society and the Norwegian Research Council 
are acknowledged. JRG is a recipient of the Canadian Association 
of Medical Oncologists/Canadian Institutes of Health Research 
Fellowship. WDF is a Chercheur Clinicien Boursier of the FRSQ.

Table 2  Cox proportional hazards model for breast cancer specific mortality

| Variable                        | Univariate analysis |                      |                      |
|---------------------------------|---------------------|----------------------|----------------------|
|                                 | RR (95% CI)         | P-value              | RR (95% CI)          | P-value |
| Tumour size (cm)                |                     |                      |                      |
| <2                              | 1.0                 |                      | 1.0                  | 0.1     |
| ≥2                              | 2.0 (1.6-4.9)       | 0.0002               | 1.6 (0.9-3.0)        | 0.04    |
| Nuclear grade                   | 2.5 (1.7-3.5)       | 0.0001               | 1.6 (1.03-2.4)       | 0.04    |
| ER status                       |                      |                      |                      |
| Positive                        | 1.0                 |                      | 1.0                  | 0.07    |
| Negative                        | 3.0 (1.8-4.8)       | 0.0001               | 1.7 (0.95-3.0)       | 0.07    |
| Lymph nodes                     |                      |                      |                      |
| Negative                        | 1.0                 |                      | 1.0                  | 0.004   |
| Positive                        | 2.5 (1.5-4.4)       | 0.0007               | 2.3 (1.3-3.4)        | 0.004   |
| Mutation carrier status         |                      |                      |                      |
| Non-carriers                    | 1.0                 |                      | 1.0                  | 0.8     |
| BRCA1 carriers                  | 1.7 (0.9-3.4)       | 0.1                  | 1.1 (0.6-2.0)        | 0.8     |
| BRCA2 carriers                  | 1.9 (0.6-6.2)       | 0.3                  |                      | 0.8     |
| BRCA1/BRCA2 carriers            | 1.8 (0.9-3.2)       | 0.07                 |                      | 0.8     |
| p53 IHC                          |                      |                      |                      |
| Negative                        | 1.0                 |                      | 1.0                  | 0.4     |
| Positive                        | 2.5 (1.5-4.1)       | 0.0003               | 1.3 (0.7-2.2)        | 0.4     |
| GMPa                            | 2.4 (1.6-3.5)       | 0.0001               | 1.9 (1.2-3.0)        | 0.006   |

ER = oestrogen receptor; GMP = glomeruloid microvascular proliferation; IHC = immunohistochemistry; MVD = microvascular density. The model was adjusted for cases with missing tumour size (n = 13) and missing lymph node status (n = 13). *As a continuous variable. BRCA1/BRCA2 carriers combined compared with non-carriers.
REFERENCES

Blackwood MA, Weber BL (1998) BRCA1 and BRCA2: from molecular genetics to clinical medicine. J Clin Oncol 16: 1969 – 1977
Chappuis PO, Goffin J, Wong N, Perret C, Ghadirian P, Tonin PN, Foulkes WD (2002) A significant response to neoadjuvant chemotherapy in BRCA1/2 related breast cancer. J Med Genet 39: 608 – 610
Dameron KM, Volpert OV, Tainsky MA, Bouck N (1994) Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. Science 265: 1582 – 1584
de Jong JS, van Diest PJ, Baak JP (2000) Hot spot microvessel density and the mitotic activity index are strong additional prognostic indicators in invasive breast cancer. Histopathology 36: 306 – 312
De Paola F, Granato AM, Scarpi E, Monti F, Medri L, Bianchi S, Amadori D, Volpi A (2002) Vascular endothelial growth factor and prognosis in patients with node-negative breast cancer. Int J Cancer 98: 228 – 233
Fisher DE (2001) The p53 tumor suppressor: critical regulator of life & death in cancer. Apoptosis 6: 7 – 15
Foulkes WD, Wong N, Brunet JS, Bégin LR, Zhang JC, Martinez JJ, Rozen F, Tonin PN, Narod SA, Karp SE, Pollak MN (1997) Germ-line BRCA1 mutation is an adverse prognostic factor in Ashkenazi Jewish women with breast cancer. Clin Cancer Res 3: 2465 – 2469
Goffin JR, Chappuis PO, Bégin LR, Wong N, Brunet JS, Hamel N, Paradis AJ, Boyd J (2003) The impact of germ-line BRCA1 mutations and over-expression of p53 on prognosis and response to treatment following breast cancer: 10 year follow up data. Cancer 97: 527 – 536
Greenblatt MS, Chappuis PO, Bond JP, Hamel N, Foulkes WD (2001) TP53 mutations in breast cancer associated with BRCA1 or BRCA2 germ-line mutations: distinctive spectrum and structural distribution. Cancer Res 61: 4092 – 4097
Hartman AR, Ford JM (2002) BRCA1 induces DNA damage recognition factors and enhances nucleotide excision repair. Nat Genet 32: 180 – 184
Linderholm B, Granqvist K, Wilking N, Johansson M, Tavelin B, Henriksson R (2000) Correlation of vascular endothelial growth factor content with recurrences, survival, and first relapse site in primary node-negative breast cancer. J Clin Oncol 18: 1423 – 1431
Mandlekar S, Kong AN (2001) Mechanisms of tamoxifen-induced apoptosis. Apoptosis 6: 469 – 477
Ravi R, Mookerjee B, Bhujwalla ZM, Sutter CH, Artemov D, Zeng Q, Dillahay LE, Madan A, Semenza GL, Bedi A (2000) Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. Genes Dev 14: 34 – 44
Straume O, Akslen LA (2001) Expression of vascular endothelial growth factor, its receptors (FLT-1, KDR) and TSP-1 related to microvessel density and patient outcome in vertical growth phase melanomas. Am J Pathol 159: 223 – 235
Straume O, Chappuis PO, Salvesen HB, Halvorson OJ, Haukaas SA, Goffin JR, Bégin LR, Foulkes WD, Akslen LA (2002) Prognostic importance of glomeruloid microvascular proliferation indicates an aggressive angiogenic phenotype in human cancers. Cancer Res 62: 6808 – 6811
Sundberg C, Nagy JA, Brown LF, Deng F, Eckelhoefer IA, Manseau EJ, Dvorak AM, Dvorak HF (2001) Glomeruloid microvascular proliferation follows adenoviral vascular permeability factor/vascular endothelial growth factor-164 gene delivery. Am J Pathol 158: 1145 – 1160
Tas F, Yavuz E, Aydiner A, Saip P, Disci R, Iplikci A, Topuz E (2000) Angiogenesis and p53 protein expression in breast cancer: prognostic roles and interrelationships. Am J Clin Oncol 23: 546 – 553
van’t Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kogel AK, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernard CS, Friend SH (2002) Gene expression profiling predicts clinical outcome of breast cancer. Nature 415: 530 – 536
Wang J, Buchholz TA, Middleton LP, Allred DC, Tucker SL, Kuerer HM, Esteva FJ, Hortobagyi GN, Sahin AA (2002) Assessment of histologic features and expression of biomarkers in predicting pathologic response to anthracycline-based neoadjuvant chemotherapy in patients with breast carcinoma. Cancer 94: 3107 – 3114
Weidner N, Folkman J, Pozza F, Bevilacqua P, Allred EN, Moore DH, Meli S, Gasparini G (1992) Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. J Natl Cancer Inst 84: 1875 – 1887
Weidner N, Semple JP, Welch WR, Folkman J (1991) Tumor angiogenesis and metastasis – correlation in invasive breast carcinoma. N Engl J Med 324: 1 – 8
Wesseling P, van der Laak JA, Link M, Teepen HL, Ruiter DJ (1998) Quantitative analysis of microvascular changes in diffuse astrocytic neoplasms with increasing grade of malignancy. Hum Pathol 29: 352 – 358
Yuan ZQ, Bégin LR, Wong N, Brunet JS, Trifiro M, Gordon PH, Pinsky L, Foulkes WD (1999) The effect of the I1307 K APC polymorphism on the risk of colorectal cancer in Italy. Int J Cancer 81: 1567 – 1571