Pergamon

European Journal of Cancer Vol. 33, No. 10, pp. 1654–1660, 1997

Printed in Great Britain
0959-8049/97 $17.00 + 0.00

PII: S0959-8049(97)00182-2

Original Paper

Expression of Osteonectin mRNA in Human Breast Tumours is Inversely Correlated with Oestrogen Receptor Content

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Osteonectin is a secreted glycoprotein which is detected in a number of normal and neoplastic human tissues *in vivo*. It is an extracellular matrix (ECM)-associated protein which is postulated to regulate cell migration, adhesion, proliferation and matrix mineralisation and previous reports suggest that it may be modulated by steroid hormones in target tissues. The aim of this study was to measure osteonectin mRNA and protein expression in breast tumour biopsies and compare these with oestrogen (ER) and progesterone receptor (PR) levels in the same tumours. An inverse correlation was seen between osteonectin mRNA expression and ER level. Samples with low ER protein expression had a mean osteonectin mRNA level which was almost 4-fold greater than the mean level of expression observed in tumours containing high concentrations of ER protein. This inverse correlation was statistically significant. Despite the strong inverse relationship between osteonectin mRNA levels and tumour ER content, no correlation was seen when osteonectin protein concentration was measured in tumour cytosols on immunoblots and compared to ER and PR levels in the same tumours. However, since it is a secreted protein, osteonectin protein expression may not reflect cellular osteonectin levels in breast tumours. In summary, these data suggest that ER-mediated suppression of osteonectin gene expression may contribute to the less aggressive characteristics associated with receptor-positive tumours and that loss of ER expression may lead to over-expression of osteonectin and contribute to a poorer differentiated, more invasive phenotype. © **1997 Elsevier Science Ltd.**

Key words: breast cancer, human biopsies, osteonectin, oestrogen receptor, RNA analysis, immunoblot analysis

Eur J Cancer, Vol. 33, No. 10, pp. 1654–1660, 1997

INTRODUCTION

OSTEONECTIN IS an extracellular matrix (ECM)-associated, secreted glycoprotein which is expressed in a variety of developing and adult tissues [1, 2]. First described in bone as a calcium-binding protein involved in bone formation and mineralisation [3], it is now known to be identical to or an interspecies homologue of SPARC (secreted protein, acidic and rich in cysteine), BM-40 and 43K culture shock protein [4] and is thought to affect tissue remodelling, wound healing and alterations in ECM interactions. Unlike many ECM-associated proteins, osteonectin does not facilitate cell attachment and spreading *in vitro*, but instead causes cell rounding and reduced numbers of focal adhesions [5]. Addition of osteonectin to culture medium causes rounding of newly plated bovine endothelial cells, fibroblasts and smooth muscle cells and prevents the normal spreading of fibroblasts and endothelial cells plated on collagen [6]. Synthesis and secretion of osteonectin is increased by changes in extracellular matrix interactions; it is induced in rat fibroblasts, *in vivo*, around the site of wound repair and also in the thrombus [7], where it is probably contributed by platelets and megakaryocytes [8, 9]. Osteonectin is also induced 20-fold during retinoic acid-induced differentiation of F9 teratocarcinoma cells, with the maximum increase accompanying a period of intense morphogenic activity [10].

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Received 9 Oct. 1996; revised 17 Mar. 1997; accepted 25 Mar. 1997.

Overexpression of osteonectin is strikingly common in a broad range of neoplastic human tissues, and it has been postulated that this may serve as a marker of disease progression or invasive potential since more intense histochemical staining has been seen in malignant cells than adjacent benign tissue [2]. Both osteonectin and stromelysin-3, a matrix-degrading metalloproteinase, are overexpressed in stromal fibroblasts of human primary colorectal cancers and their liver metastases, compared with normal mucosa. Increased osteonectin mRNA and protein expression is observed at early stages of the disease, whereas stromelysin-3 induction is correlated with disease progression [11]. Recent studies have shown that osteonectin and another matrix protein, osteopontin, are overexpressed in malignant breast tumours compared with normal tissue or benign lesions [12, 13], but it is not known whether their overexpression is related to expression of biological markers such as steroid hormone receptors.

Several lines of evidence support a role for steroid hormones in regulating the expression and function of matrix proteins and metalloproteinases. Oestrogen is known to influence gender-specific bone formation during development and to decrease bone remodelling and osteopenia [14, 15], whereas glucocorticoids have been implicated in the process of bone loss [16–18]. Strong evidence exists for progestin regulation of tissue remodelling via metalloproteinases [19, 20]. Therefore, the hormonal environment is important in the control of matrix formation and remodelling and, by inference, the expression of steroid hormone receptors is likely to influence the functional activity of matrix proteins in a given tissue. Approximately 80% of breast tumours contain oestrogen receptors (ER) [21]. In breast cancers, the presence of ER is associated with good prognosis, and a greater likelihood of response to endocrine treatment in cases of advanced disease, this being strengthened by the concomitant expression of progesterone receptors (PR) [21]. Given the significance of ER expression in breast tumours and the likely role of steroid hormones in the regulation of osteonectin expression, the relationship between osteonectin expression and the expression of ER and PR in ER positive human breast tumours was determined.

MATERIALS AND METHODS

Materials

Chemicals were of analytical reagent grade and were obtained from the sources listed previously [22]. Anti-osteonectin monoclonal antibodies were obtained (lyophilised) from Biodesign International (ME, U.S.A.) and reconstituted to 1 mg/ml. Abbott PgR-EIA Monoclonal and ER-EIA Monoclonal reagents for enzyme immunoassay (EIA) were from Abbott Laboratories (Diagnostics Division, Illinois, U.S.A.). The osteonectin cDNA used to prepare labelled probes was obtained by screening a human uterus cDNA-λgt-11 library (Clontech, California, U.S.A.). A 1557 bp insert, corresponding to positions +561 to +2118 of the published cDNA sequence [23], was purified from a positive recombinant plaque and subcloned into the EcoRI site of pGEM-7Zf(+) (Promega, Australia) and verified by sequencing (not shown).

RNA preparation and Northern blot analysis

Biopsy samples ($n = 17$) were collected at the time of breast lump excision and frozen at −70°C. The tissues were pulverised in a precooled vessel, then suspended in a guanidinium isothiocyanate solution and homogenised using a Dounce homogeniser and teflon pestle. Total RNA was isolated by the guanidinium isothiocyanate-caesium chloride method, and Northern blot analysis was carried out as described previously [22] except that the cDNA probe was labelled by random priming using the Amersham Megaprime DNA labelling system (Amersham, Australia). Sources, tissue culture and Northern blot analysis of established cell lines were as previously described [24]. The cell lines used were ER+: MCF-7, MCF-7M, MCF-7Z, T-47D, MDA-MB-361, MDA-MB-134, and ER-: MDA-MB-231, MDA-MB-453, BT-20 human breast cancer cells, ER-HBL-100 transformed breast epithelial cell line and ER-LNCaP prostate carcinoma cell line. Differences in loading were normalised by quantitation of 18 s rRNA in each sample. This was detected using a 20 bp oligonucleotide homologous to the 18 s cDNA sequence, labelled using T4-polynucleotide kinase (Bresatec, Australia) and $\gamma[^{32}P]$-ATP (Amersham, Australia). Osteonectin and 18 s RNA signals were detected on Northern blots by exposure to a phosphor screen and scanned and quantitated using a Molecular Dynamics phosphorimager and Imagequant software. An apparent minor band migrating with slower mobility compared with the major osteonectin transcript appeared to be the result of compression of the trailing edge of the specific osteonectin band by the 28 s rRNA subunit and was included in quantitation.

Protein cytosol preparation and immunoblot analysis

Tissues which had been frozen at −70°C ($n = 44$) were pulverised in a precooled vessel, then homogenised at 4°C in a buffer containing 10mM Tris pH 7.4–7.5, 1.5 mM EDTA 5mM sodium molybdate and 1mM monothioglycerol. Cytosols were prepared by centrifugation at 436 000 *g* for 20 min at 4°C. Protein concentrations were determined by a method based on that of Lowry and associates [25] as recommended in notes accompanying the Abbott EIA reagents. PR and ER concentrations were determined by EIA. The cytosols were stored at −80°C.

Tumour cytosol proteins (150 μg per lane) were separated by electrophoresis through 10% polyacrylamide gels containing SDS and electroblotted overnight on to nitrocellulose as previously described [22]. Osteonectin protein was detected using anti-osteonectin monoclonal antibody at a dilution of 1:3000. Specific osteonectin protein was visualised on autoradiographic film using a horseradish peroxidase conjugated sheep anti-mouse secondary antibody (Silenus Laboratories, Australia) at 1:3000 dilution and ECL chemiluminescent detection substrates (Amersham, Australia). A cytosol (50 μg and 100 μg) made from the SaOS-2 osteosarcoma cell line which expresses osteonectin [9] was included on each gel as a positive control. Band intensities were measured densitometrically using a Molecular Dynamics densitometer and Imagequant software. The linear range of quantitation of chemiluminescent signals was previously determined using a standard curve of increasing amounts of a known positive control signal [26]. Several different exposure times were performed with each immunoblot and osteonectin signals, in tumour samples and controls, were

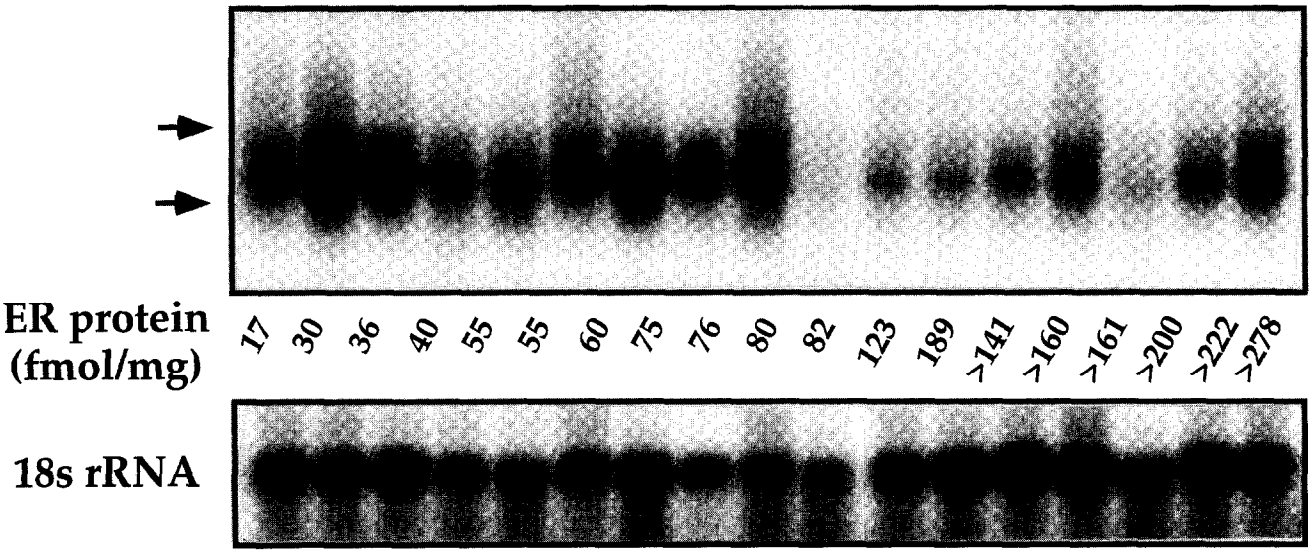

Figure 1. Detection of osteonectin mRNA in human breast tumours. 20 μg RNA from each sample was analysed by Northern blot as described in Materials and Methods. Osteonectin mRNA was detected using a random primed cDNA probe (upper panel) and 18 s rRNA was visualised later on the same blot using a ^{32}P end-labelled oligonucleotide. Arrows indicate the positions of the 18 s and 28 s ribosomal subunits visualised on the Northern blot with ethidium bromide staining. The concentration of ER protein in each sample, estimated by EIA, is indicated below each lane. Where ER concentration was higher than the upper limit of the standard curve the maximum standard value is shown, with a > symbol, as an estimate of the ER concentration in those samples.

quantitated from exposures which fell within the linear range and data corrected for loading using the positive control from the same exposure.

To allow correlation of osteonectin concentration with PR and ER EIA, tumour osteonectin protein levels were normalised to osteonectin concentration in the SaOS-2 positive control cytosol. This was done by dividing the immunoreactive osteonectin protein (AU) in the tumour sample by the immunoreactive osteonectin (AU) per microgram SaOS-2 cytosol from the same immunoblot. Linear regression,

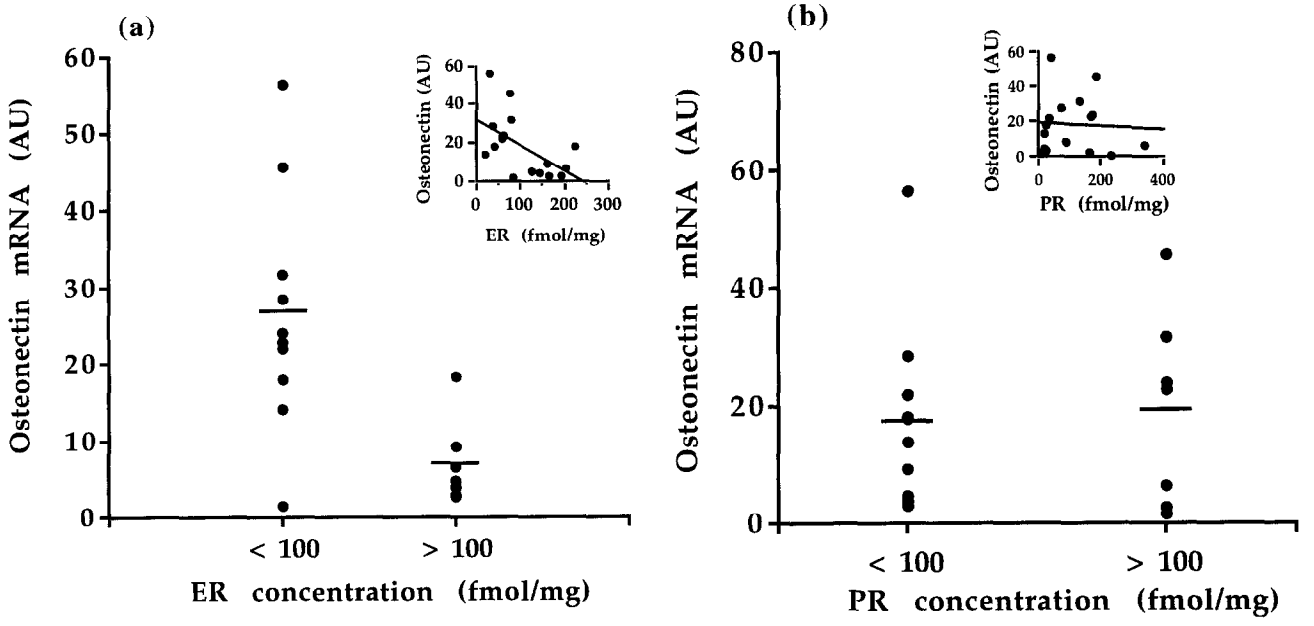

Figure 2. Comparison of oesteonectin mRNA concentration with (a) ER protein in human breast tumours. Samples were divided between ER concentrations above or below 100 fmol/mg tumour protein for comparison with osteonectin mRNA. Osteonectin mRNA levels were compared between the two ER concentration groups by student's *t*-test ($P = 0.0066$). Inset. Osteonectin mRNA levels were compared to ER protein concentrations, estimated in the same manner as described in the legend to Figure 1, in the same tumours by linear regression ($P = 0.0095$). (b) PR protein in human breast tumours. Samples were divided between PR concentrations above or below 100 fmol/mg tumour protein for comparison with osteonectin mRNA. Osteonectin mRNA levels were compared between the two PR concentration groups by student's *t*-test ($P = 0.8534$). Inset. Osteonectin mRNA levels were compared to PR protein concentrations, estimated by EIA, in the same tumours by linear regression. Where PR was higher than the upper limit of the standard curve of the assay, the maximum standard value was taken as an estimate of the PR concentration in those samples. Horizontal bars indicate means.

analysis of variance and student's *t*-test analyses were carried out using StatView (Abacus Concepts Inc., California, U.S.A.) statistical analysis software.

RESULTS

Expression of osteonectin mRNA was measured by Northern blot analysis of total RNA from 17 human breast tumour biopsies (Figure 1). The osteonectin transcript was detected migrating slightly behind the 18 s ribosomal subunit, with predicted size of 3 kb. This was consistent with previous characterisation of the osteonectin transcript [23]. A 2.2 kb transcript has been reported in some tissues, arising from alternative polyadenylation, but this transcript was not detected in the breast tumours examined. Osteonectin mRNA was detected in all 17 of the breast tumours to differing levels and was quantitated densitometrically and normalised for loading using 18 s rRNA.

When osteonectin mRNA expression was compared to ER protein level, a statistically significant inverse correlation was observed (Figure 2; $P = 0.0095$). Osteonectin mRNA detection levels fell within a range of 1.77–56.6 AU, after normalisation for loading. The mean osteonectin level in tumours with ER levels less than 100 fmol/mg was 26.6 ± 15.5 (S.D.) and in tumours containing greater than 100 fmol ER/mg was a 7.1 ± 5.5. The difference between these groups was significant by the student's *t*-test ($P = 0.0066$). No correlation was observed with PR expression ($P = 0.8543$, Figure 2). The mean osteonectin mRNA levels in tumours with PR concentrations less than 100 fmol/mg compared with those greater than 100 fmol/mg were 17.98 ± 15.92 and 19.48 ± 16.45, respectively.

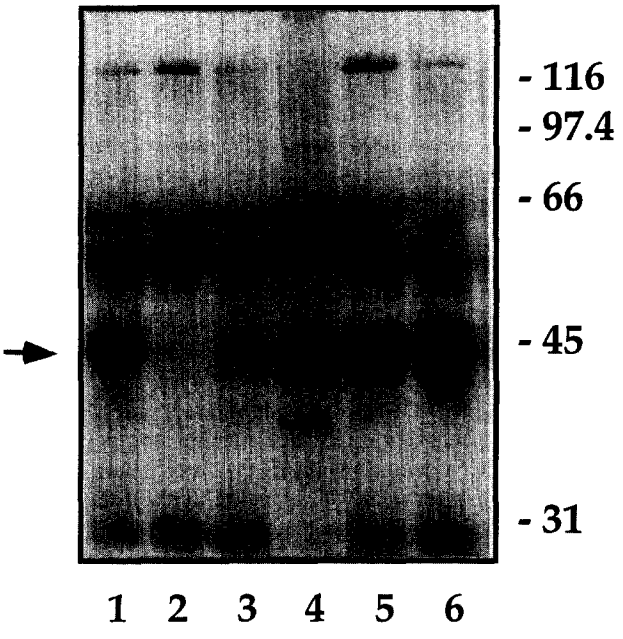

Figure 3. Detection of osteonectin protein. Cytosol proteins (150 μg; lanes 1–3, 6) were separated on 10% polyacrylamide–SDS gels with a SaOS-2 cell line positive control cytosol (100 μg, lane 4) and 150 μg cytosol from bone metastasis of breast tumour origin (lane 5) and transferred to nitrocellulose. Osteonectin protein was visualised using an anti-osteonectin MAb and chemiluminescent detection. The arrow indicates specific osteonectin protein bands. The mobilities of protein molecular weight markers are indicated on the right.

Limitations of the ER EIA caused underestimation of ER in some samples where ER was very high and therefore outside the upper limit of the standard curve. However, if regression analysis was carried out between osteonectin mRNA and estimated ER concentration, taking the maximum standard values as representing the ER concentration in those samples and recognising that these were underestimated values, a statistically significant inverse correlation was still observed between the two parameters ($P = 0.0209$, Figure 2, inset). This contrasted with the comparison of osteonectin mRNA to PR concentrations where no relationship was seen (Figure 2, inset). No difference was seen between osteonectin levels in ER + PR+ tumours and those that were ER + PR- (data not shown).

To determine whether the inverse correlation between osteonectin transcript expression and ER concentration was reflected at the protein level, breast tumour cytosols were examined for expression of osteonectin. Osteonectin was detected as an approximately 43 kDa protein on immunoblots and a representative immunoblot is shown in Figure 3. A cytosol made from the SaOS-2 osteosarcoma cell line (Figure 3, lane 4) was run as a positive control for quantitation, and a cytosol from a bone metastasis of breast tumour origin (Figure 3, lane 5) was also included on each gel. A less intense band was detected below the major immunoreactive band in the SaOS-2 cytosol (Figure 3, lane 4). This may reflect the presence of differentially glycosylated osteonectin forms in SaOS-2 cells, since these cells have previously been reported to synthesise and secrete distinct forms of the protein [9]. The mobility of the major osteonectin band in the SaOS-2 appeared to be slightly slower than that of the protein detected in the bone and breast tumour cytosols. This has also been observed previously as a difference in post-translational processing of the protein in this cell line versus tumour tissue [9]. The amount of osteonectin protein detected varied considerably between tumours and was very low or absent in a number of samples. This did not appear to be due to protein degradation since no smaller immunoreactive proteins were detected.

Osteonectin protein was quantitated in each tumour by densitometry and each sample was normalised to SaOS-2 osteonectin level from the same immunoblot to control for differences in transfer. This was done by dividing tumour osteonectin (AU) by the level of osteonectin per microgram of SaOS-2 cytosol (AU) on each gel. Osteonectin concentrations in the samples ranged between 16.17 and 166.76 AU. Despite the strong relationship observed between osteonectin mRNA and ER, no correlation was found between osteonectin protein and ER concentration when examined by analysis of variance ($P = 0.2569$, Figure 4). No significant difference in osteonectin protein level was seen by student's *t*-test between samples with ER less than 100 fmol/mg compared to those with ER greater than 100 fmol/mg (data not shown). No correlation was seen when estimated ER concentrations were compared to osteonectin protein levels ($P = 0.2268$, data not shown). Furthermore, when the 21 ER+ samples were compared with 23 tumours which were ER-, no significant difference was observed. The results of statistical analyses of osteonectin mRNA and protein levels in the tumour samples are summarised in Table 1.

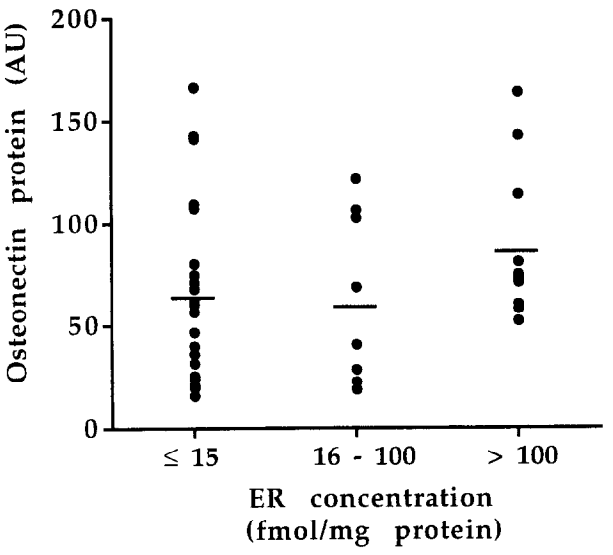

Figure 4. Comparison of osteonectin and ER protein concentrations in human breast tumours. Osteonectin protein levels were compared to ER levels (estimated by EIA) in the same samples, grouped into ranges of ER concentration, by analysis of variance ($P = 0.2569$).

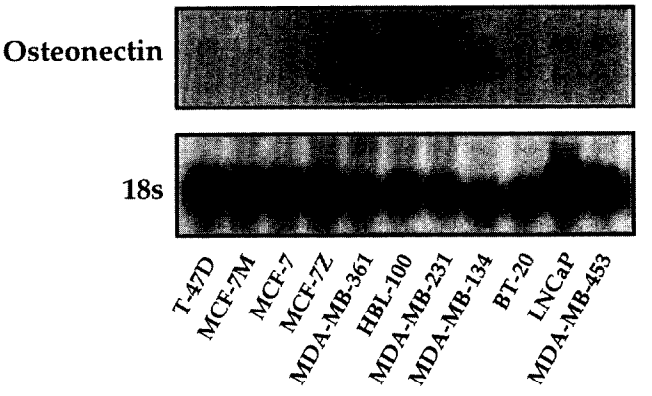

Figure 5. Osteonectin mRNA expression in cell lines. Total RNA isolated from 9 breast cancer cell lines (ER+: T-47D, MCF-7, MCF-7M, MCF-7Z, MDA-MB-361, MDA-MB-134 and ER−: MDA-MB-231, MDA-MB-453, BT-20), HBL-100 transformed breast epithelial cells (ER−) and the LNCaP prostate carcinoma cell line (ER-) by the guanidinium isothiocyanate–caesium chloride method was analysed by Northern blotting (20 μg/sample) as described in Materials and Methods. The blot was probed sequentially for osteonectin mRNA and 18 s rRNA expression.

To examine whether the relationship between osteonectin mRNA expression and ER status was observed in breast cancer cell lines, its expression was examined in ER+ and ER- human breast cancer cell lines, the HBL-100 transformed breast epithelial cell line and LNCaP prostate carcinoma cells. Osteonectin mRNA and 18 s rRNA expression were examined in total RNA from each cell line by Northern blot analysis and the results are shown in Figure 5. Osteonectin mRNA expression was not detectable in any cell line except HBL-100, where it was detected with the same mobility as in the tumours.

DISCUSSION

Osteonectin mRNA expression was examined in 17 human primary breast tumour samples and was measurable in every case. Quantitation of the osteonectin signal and comparison with ER and PR levels revealed a statistically significant inverse correlation with ER level. The expression of ER in breast cancer is associated with good clinical prognosis. The presence of ER in primary breast tumours is associated with a longer disease-free interval from the time of initial diagnosis and treatment [27]. Furthermore, expression of ER is inversely proportional to the proliferation rate, suggesting that ER+ tumours are often better differentiated and less aggressive [28]. Conversely, osteonectin expression is postulated to be associated with malignant progression and invasive potential in colorectal cancer [11], and in breast cancer [2, 12, 13].

Table 1. Osteonectin mRNA and protein levels in human breast tumours

ER (fmol/mg)	Osteonectin mRNA n	Osteonectin mRNA (mean ± SD)*	Osteonectin protein n	Osteonectin protein (mean ± SD)†
0–15	0		23	63.9 ± 43.4
16–100	10	26.6 ± 15.5	9	59.5 ± 41.4
>100	7	7.1 ± 5.5	12	85.7 ± 35.8

*AU, range = 1.77–56.6, $P = 0.0066$, student's t-test for difference between groups. †AU, range = 16.17–166.76, $P = 0.2569$, analysis of variance between the groups. n = number of tumour samples in each group. Range = range of osteonectin mRNA or protein levels estimated in AU.

The demonstration in this study that tumours with low ER levels are more likely to express osteonectin is consistent with the postulated role of osteonectin in malignant progression. Bellahcene and Castronovo [12] postulated that the affinity of osteonectin for type I collagen of bone matrix may enhance the homing of osteonectin-rich metastatic breast tumour cells to bone, a relatively common metastatic site of breast cancers. However, this remains to be demonstrated and a number of alternative mechanisms of osteonectin involvement in tumour invasiveness have been suggested. Osteonectin has been shown to increase the expression of metalloproteinases in cultured fibroblasts, including collagenase and stromelysin-3, which are involved in the breakdown of interstitial and basement membrane matrices and, therefore, could facilitate the escape of tumour cells from the surrounding ECM [29]. This is supported by reports that stromelysin-3 is overexpressed in breast and colorectal cancers and is correlated with disease stage and degree of invasiveness [11, 30, 31]. Furthermore, osteonectin has been shown *in vitro* to inhibit cell adhesion and spreading on ECM protein monolayers and to promote cell rounding, suggesting that osteonectin may decrease the number of ECM adhesion points by interference with the cell plasma membrane as well as by inducing ECM protein-degrading metalloproteinases [6].

The correlation observed between osteonectin mRNA and ER expression was not seen at the protein level on immunoblots of 44 breast tumour cytosols. The reason for this difference is not clear. If there is no relationship between ER levels and osteonectin protein expression, it may indicate that osteonectin protein expression is of little significance in tumour progression. However, given that osteonectin is a secreted protein, differences in the rate of secretion of the protein between tumours may complicate the quantitation of cellular osteonectin concentration. Clarification of the relationship between osteonectin mRNA

and protein expression is required to address this issue. Furthermore, the relative location of osteonectin mRNA and protein expression was not examined in breast tumours in this study, although in colorectal cancers osteonectin mRNA is detected in the stroma [11].

It was surprising to note that little or no expression of osteonectin mRNA was seen in breast cancer cell lines. This was contrary to the positive expression seen in the breast tumour samples in this study and also to consistent reports of elevated expression of osteonectin in neoplastic tissues *in vivo*, including malignant breast epithelium [2, 11, 12]. Osteonectin expression may require the association of epithelial cells *in vivo* with adjacent stroma, an association which is lost in breast cancer cells in culture. This may explain the lack of osteonectin expression in the cell lines tested, which are largely derived from metastatic breast cancers.

There is strong evidence that matrix proteins and metalloproteinases are regulated by steroid hormones. Oestrogen downregulation of mRNAs for bone matrix-associated proteins, including osteonectin, has been reported to decrease bone resorption and formation, resulting in a net slowing of the rate of loss of bone mass [14, 15]. Glucocorticoids have been implicated in the process of bone loss through their ability to block $1,25(OH)_2$-vitamin D-induced osteocalcin synthesis [16] and to prevent attachment of osteoblasts to matrix proteins, including osteonectin, possibly through downregulation of β1-integrin and other cell-surface attachment factors [17]. Glucocorticoids also increase bone sialoprotein mRNA levels in rat osteosarcoma cells, an effect which can be blocked by $1,25(OH)_2$-vitamin D and has been postulated to contribute to acceleration in maturation of preosteoblasts and ultimately to contribute to bone loss [18]. Progestins are postulated to regulate tissue remodelling via metalloproteinases. Progestins suppress expression of stromelysins in endometrial stromal cells and induce TGF-β in these cells, resulting in downregulation of matrilysin expression in the endometrial epithelium of stromal–epithelial cocultures [19, 20]. Furthermore, the demonstration that a sequence contained in the 5′-flanking region of the mouse osteonectin gene can act as a progestin response element *in vitro* suggests that progestins may also regulate this protein *in vivo* [32]. Given that oestrogen and progesterone suppress the expression of osteonectin and metalloproteinases in normal tissues [14, 15, 19, 20], it is possible that lack of ER and PR expression in breast tumours may lead to a loss of expression of these factors. This may be one mechanism involved in tumour progression and the acquisition of a greater invasive potential.

In summary, this study demonstrates a correlation between lack of ER expression and high expression of osteonectin mRNA in breast cancer. Loss of regulation of matrix protein expression in this tissue may contribute to the acquisition of a more aggressive phenotype in breast tumours.

1. Lane TF, Sage EH. The biology of SPARC, a protein that modulates cell–matrix interactions. *FASEB J* 1994, **8**, 163–173.
2. Porter PL, Sage EH, Lane TF, Funk SE, Gown AM. Distribution of SPARC in normal and neoplastic human tissue. *J Histochem Cytochem* 1995, **43**, 791–800.
3. Termine JD, Kleinman HK, Whitson SW, Conn KM, McGarvey ML, Martin GR. Osteonectin, a bone-specific protein linking mineral to collagen. *Cell* 1981, **26**, 99–105.
4. Mann KG, Deutzmann R, Paulsson M, Timpl R. Solubilization of protein BM-40 from a basement membrane tumour with chelating agents and evidence for its identity with osteonectin and SPARC. *FEBS Lett* 1987, **218**, 167–172.
5. Sage EH, Bornstein P. Extracellular proteins that modulate cell–matrix interactions. SPARC, tenascin and thrombospondin. *J Biol Chem* 1991, **266**, 14831–14834.
6. Sage H, Vernon RB, Funk SE, Everitt EA, Angello J. SPARC, a secreted protein associated with cellular proliferation, inhibits cell spreading *in vitro* and exhibits Ca^{2+}-dependent binding to the extracellular matrix. *J Cell Biol* 1989, **109**, 341–356.
7. Reed MJ, Puolakkainen P, Lane TF, Dickerson D, Bornstein P, Stage EH. Differential expression of SPARC and thrombospondin 1 in wound repair: immunolocalization and *in situ* hybridization. *J Histochem Cytochem* 1993, **41**, 1467–1477.
8. Stenner DD, Tracy RP, Riggs BL, Mann KG. Human platelets contain and secrete osteonectin, a major protein of mineralized bone. *Proc Natl Acad Sci USA* 1986, **83**, 6892–6896.
9. Kelm RJ Jr, Hair GA, Mann KG, Grant BW. Characterization of human osteoblast and megakaryocyte-derived osteonectin (SPARC). *Blood* 1992, **80**, 3112–3119.
10. Mason IJ, Murphy D, Munke M, Franke U, Elliott RW, Hogan BLM. Developmental and transformation-sensitive expression of the Sparc gene on mouse chromosome 11. *EMBO J* 1986, **5**, 1831–1837.
11. Porte H, Chastre E, Prevot S, *et al.* Neoplastic progression of human colorectal cancer is associated with overexpression of the stromelysin-3 and BM-40/SPARC genes. *Int J Cancer* 1995, **64**, 70–75.
12. Bellahcene A, Castronovo V. Increased expression of osteonectin and osteopontin, two bone matrix proteins, in human breast cancer. *Am J Pathol* 1995, **146**, 95–100.
13. Hirota S, Ito A, Nagoshi J, *et al.* Expression of bone matrix protein messenger ribonucleic acids in human breast cancers. Possible involvement of osteopontin in development of calcifying foci. *Lab Invest* 1995, **72**, 64–69.
14. Turner RT, Colvard DS, Spelsberg TC. Estrogen inhibition of periosteal bone formation in rat long bones: down-regulation of gene expression for bone matrix proteins. *Endocrinology* 1990, **127**, 1346–1351.
15. Turner RT, Backup P, Sherman PJ, Hill E, Evans GL, Spelsberg TC. Mechanism of action of oestrogen on intramebranous bone formation: regulation of osteoblast differentiation and activity. *Endocrinology* 1992, **131**, 883–889.
16. Beresford JN, Gallagher JA, Poser JW, Russell RGG. Production of osteocalcin by human bone cells *in vitro*. Effects of 1,25(OH)2D3, parathyroid hormone, and glucocorticoids. *Metab Bone Dis Rel Res* 1984, **5**, 229–234.
17. Gronowicz GA, McCarthy M-B. Glucocorticoids inhibit the attachment of osteoblasts to bone extracellular matrix proteins and decrease β1-integrin levels. *Endocrinology* 1995, **136**, 598–608.
18. Oldberg A, Jirskog-Hed B, Axelsson S, Heinegard D. Regulation of bone sialoprotein mRNA by steroid hormones. *J Cell Biol* 1989, **109**, 3183–3186.
19. Osteen KG, Rodgers WH, Gaire M, Hargrove JT, Gorstein F, Matrisian LM. Stromal-epithelial interaction mediates steroidal regulation of metalloproteinase expression in human endometrium. *Proc Natl Acad Sci USA* 1994, **91**, 10129–10133.
20. Bruner KL, Rodgers WH, Gold LI, *et al.* Transforming growth factor beta mediates the progesterone suppression of an epithelial metalloproteinase by adjacent stroma in the human endometrium. *Proc Natl Acad Sci USA* 1995, **92**, 7362–7366.
21. McGuire WL, Chamness GC, Fuqua SAW. Estrogen receptor variants in clinical breast cancer. *Mol Endocrinol* 1991, **5**, 1571–1577.
22. Clarke CL, Roman SD, Graham J, Koga M, Sutherland RL. Progesterone receptor regulation by retinoic acid in the human breast cancer cell line T-47D. *J Biol Chem* 1990, **265**, 12694–12700.
23. Swaroop A, Hogan BLM, Franke U. Molecular analysis of the cDNA for human SPARC/osteonectin/BM-40: sequence, expression, and localization of the gene to chromosome 5q31-q33. *Genomics* 1988, **2**, 37–47.

24. Roman SD, Clarke CL, Hall RE, Alexander IE, Sutherland RL. Expression and regulation of retinoic acid receptors in human breast cancer cells. *Cancer Res* 1992, **52**, 2236–2242.
25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurements with Folin-phenol reagent. *J Biol Chem* 1951, **193**, 265–275.
26. Graham JD, Yeates C, Balleine RL, *et al.* Characterization of progesterone receptor A and B expression in human breast cancer. *Cancer Res* 1995, **55**, 5063–5068.
27. Knight WA, Livingston RB, Gregory EJ, McGuire WL. Estrogen receptor as an independent prognostic factor for early recurrence in breast cancer. *Cancer Res* 1977, **37**, 4669–4671.
28. Meyer JS, Rao BR, Stevens SC, White WL. Low incidence of oestrogen receptor in breast carcinomas with rapid rates of cellular replication. *Cancer* 1977, **40**, 2290–2298.
29. Tremble PM, Lane TF, Sage EH, Werb Z. SPARC, a secreted protein associated with morphogenesis and tissue remodeling, induces expression of metalloproteinases in fibroblasts through a novel extracellular matrix-dependent pathway. *J Cell Biol* 1993, **121**, 1433–1444.
30. Hähnel E, Harvey JM, Joyce R, Robbins PD, Sterrett GF, Hähnel R. Stromelysin-3 expression in breast cancer biopsies: clinico-pathological correlations. *Int J Cancer* 1993, **55**, 771–774.
31. Hähnel E, Dawkins H, Robbins P, Hähnel R. Expression of stromelysin-3 and nm23 in breast carcinoma and related tissues. *Int J Cancer* 1994, **58**, 157–160.
32. Nomura S, Hashmi S, McVey JH, Ham J, Parker M, Hogan BLM. Evidence for positive and negative regulatory elements in the 5′-flanking sequence of the mouse sparc (osteonectin) gene. *J Biol Chem* 1989, **264**, 12201–12207.

Acknowledgements—This work was supported by grants from the National Health and Medical Research Council (NHMRC) of Australia and the NSW Cancer Council. J.D. Graham is supported by an NHMRC Dora Lush Biomedical Scholarship and by the Westmead Hospital Research Institute. R.L. Balleine is supported by an NHMRC Medical Scholarship. The authors would like to thank the Department of Haematology, Institute of Clinical Pathology and Medical Research, Westmead Hospital and the Department of Radiation Oncology, Westmead Hospital for their support.