Reduced Expression of the Small Leucine-rich Proteoglycans, Lumican, and Decorin Is Associated with Poor Outcome in Node-negative Invasive Breast Cancer[1]

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ABSTRACT

Purpose: To examine the prognostic significance of lumican and decorin, two abundant small leucine-rich proteoglycans in breast tissue stroma.

Experimental design: Lumican and decorin expression was examined in a cohort of 140 invasive breast carcinomas by Western blot analysis. All cases were axillary lymph node-negative and treated by adjuvant endocrine therapy.

Results: Lumican and decorin expression was highly correlated ($r = 0.45$, $P < 0.0001$), but although low levels of lumican were associated with large tumor size ($P = 0.0496$), negative estrogen receptor ($P = 0.0024$) and progesterone receptor status ($P = 0.0116$), and increased host inflammatory response ($P = 0.0077$), low decorin levels were associated only with large tumor size ($P = 0.0496$). However, using univariate analysis, low levels of lumican and decorin were both associated with a shorter time to progression ($P = 0.0013$ and 0.0262) and poorer survival ($P = 0.001$ and 0.0076). In multivariate analysis using the Cox regression model, low decorin was also shown to be an independent predictive factor for recurrence (hazard ratio 2.25: 95% confidence interval 1–5, $P = 0.047$) and survival (hazard ratio 3.39: 95% confidence interval 1.2–9.6, $P = 0.021$).

Conclusions: These results suggest that low levels of small leucine-rich proteoglycans in breast tumors may be associated with a worse prognosis in lymph node-negative invasive breast carcinomas and warrant further study with larger patient cohorts.

INTRODUCTION

The management of ductal carcinoma *in situ* and early invasive carcinoma depends on the estimation of the biological potential for progression. However, established indicators such as nodal status and tumor size are now lacking as discriminators of low and high risk of progression, and treatment decisions must rest on tissue-based morphological and biological markers. Recent morphological studies have provided useful improvements to older classifications and grading of preinvasive disease; however, there is clearly a need for better molecular predictors of biological potential for progression in early lesions (1, 2).

The development and capacity for invasiveness is a critical biological event in progression (3). Among proposed "invasion"-related genes expressed by tumor cells are cell adhesion molecules, proteases, and cytoskeletal molecules that may influence motility. However, stromal changes and genes expressed by host stromal cells may also play an important role in tumor invasion.

The normal stroma is composed of a variety of different proteins derived largely from fibroblasts. These are known to include collagens, glycoproteins, glycosaminoglycans, and several proteoglycans (hyaluronan, perlecan, and versican; Ref. 4). The most prominent of these components in determining breast stromal architecture are the fibrillar collagens. These are secreted as triple helical procollagen molecules that undergo extracellular processing and assembly into collagen fibrils followed by cross-linking and aggregation to form collagen fibers. This extracellular processing is dependent on at least three enzymes, including procollagen proteinases and the cross-linking enzyme lysyl oxidase (5, 6), but fibrillogenesis and fibril spacing are also affected by a number of additional structural proteins and proteoglycans (7). Among these are members of the SLRP[3] gene family (8).

In breast cancer, as in many solid tumors, significant alterations to stromal structure and composition have long

[3] The abbreviations used are: SLRP, small leucine-rich proteoglycan; EGFR, epidermal growth factor receptor; ER, estrogen receptor; TGF, transforming growth factor; ECM, extracellular matrix; PR, progesterone receptor; ns, not significant.

been recognized. However, the role of this "stromal reaction" in modulating progression of invasive tumors is unresolved. We have recently identified lumican as an mRNA that is consistently differentially expressed between normal and neoplastic stroma with highest levels adjacent to *in situ* components and at the invasive edge (9). In a subsequent study of SLRP expression in breast tumors, we have also found that lumican and decorin are the most abundant SLRPs and frequently expressed, whereas biglycan and fibromodulin are only occasionally detected (10). Although the role for altered lumican expression in tumors has not been considered previously, decorin has been studied in other tumor types. An early view was that increased expression of decorin might facilitate tumor growth (11, 12). However, more recent data concerning decorin's inhibitory effects on tumor cell growth (13, 14) support examination of the alternative view that reduced decorin may facilitate tumorigenesis and growth. However, the relationship between expression of either SLRP and outcome has not been studied in breast cancer. We have therefore set out to examine whether reduced expression of lumican or decorin is associated with patient survival.

MATERIALS AND METHODS

Tissues. All breast tumor cases used for this study were selected from the Manitoba Breast Tumor Bank (Winnipeg, Manitoba, Canada; Ref. 15), which operates with the approval from the Faculty of Medicine, University of Manitoba, Research Ethics Board. As has been described previously, tissues are accrued to the bank from cases at multiple centers within Manitoba, rapidly collected, and processed to create matched, formalin-fixed, paraffin-embedded, and frozen tissue blocks with the mirror image surfaces oriented by colored inks. The histology and cellular composition of every sample in the bank is interpreted in H&E-stained sections from the face of the former tissue block.

Clinical-pathological Characteristics of the Patient Cohort. To select a study cohort, we reviewed the first 1000 cases of invasive breast cancer with complete primary clinical data and follow-up in the Manitoba Breast Tumor Bank database and identified among these all cases associated with node-negative status that were treated by surgery with or without radiation therapy and then tamoxifen endocrine therapy. Of these, 140 cases of invasive breast cancer were selected for analysis of lumican and decorin expression on the basis of: (*a*) minimum follow-up duration of 6 months; and (*b*) sufficient high-quality tissue from central regions of tumor remaining and available in the bank. Tissue sections from the mirror image paraffin block to the frozen block used for these assays have been assessed for tumor grade and inflammation. The clinical-pathological characteristics of the final study case series are shown in Table 1, including the full cohort of 140 cases that was studied for SLRP expression and a subcohort of 70 cases that was also studied for EGFR. Specific criteria for interpretation of the variables was as follows: (*a*) ER levels of >3 fmol/mg protein and PR levels of >15 fmol/mg protein were considered positive; (*b*) grade, determined by the Nottingham system, was assigned to low (scores 3–5), moderate (scores 6 and 7), or high (scores 8 and 9) categories; (*c*) tumor size, measured in centi-

Table 1 Clinical-pathological characteristics of the study cohort

Prognostic factor		SLRP cohort		EGFR cohort	
		No.	%	No.	%
ER	−ve	15	11	13	19
	+ve	125	89	57	81
PR	−ve	44	31	26	37
	+ve	96	69	44	63
EGFR	Low			35	50
	High			35	50
Grade	Low	47	34	25	42
	Int	67	48	31	44
	High	26	18	10	14
Size	≤2 cms	47	34	19	27
	>2 cms	93	66	51	73
INFL[a]	Low	121	86	57	81
	High	19	14	13	19
Type	Ductal	97	70	43	61
	Lobular	17	12	12	17
	Ductal mixed	13	9	7	11
	Special type	13	9	8	11
Age	≤50	16	11	8	11
	>50	124	89	62	89

[a] INFL, inflammation.

meters, was assigned either small (≤2 cm) or large (>2cm) categories; and (*d*) tumor inflammation/immune response was assessed in the tumor tissue section by a subjective scale from 1 to 5 and then assigned to low (score 1–3) or high (score 4 and 5) categories.

SDS-PAGE and Immunoblotting. Total proteins were extracted from frozen tissue sections, and three separate Western blots were performed using total proteins from each case to analyze the expression of lumican, decorin, and EGFR. Sections were cut from the face of frozen tissue blocks immediately adjacent to the face of a matching paraffin block from which paraffin sections had been cut previously for pathological assessment. An average of 20 20-μm tissue sections was cut from each typical tissue block (0.5 × 1 cm^2 cross-sectional area) and used for extraction; however, the number of tissue sections was varied for each case according to the measured area of the tissue within individual blocks to ensure that equivalent volumes of tissue were used for the extraction. Frozen tissues were homogenized in ice-cold homogenization buffer [20 mM 4-morpholinepropane-sulfonic acid (pH 7.2–7.5), 60 mM β-glycerophosphate, 5 mM EGTA (pH 8.0), 5 mM sodium fluoride, 1 mM sodium vanadate, and 1% NP40 (all from Sigma Chemical Co.)] and mini-protease inhibitor cocktail tablet (Boehringer Mannheim) per 10-ml extraction buffer and sonicated for 5 s several times using an ultrasonic cell disrupter (Sonics & Materials, Inc., Danbury, CT). Sonicates were centrifuged at 13,000 rpm for 20 min at 4°C and stored at −20°C until use. Protein concentrations for each case sample were determined using Bio-Rad Protein Assay, and equal amounts (50 μg) of total proteins were analyzed by SDS-PAGE and immunoblotting. Antipeptide antibodies specific for the COOH-terminal regions of the core proteins of lumican and decorin were used (16). The specificity of both antibodies was verified previously by peptide absorption and SLRP cross-reactivity anal-

ysis (16). Anti-EGFR antibody was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Supernatant samples containing 50 μg of total protein were mixed 1:1 with sample buffer [1.25 mm Tris-HCl (pH 6.8), 20% glycerol, 4% SDS, 0.02% (w/v) Bromphenol Blue, and 20 mm DTT], boiled for 5 min, electrophoresed using a 4% stacking gel and either a 10% (lumican and decorin) or 7.5% (EGFR) resolving poly-acrylamide gel, and electrophoretically transferred to 0.45-μm polyvinylidene difluoride membranes (Boehringer Mannheim). Membranes were blocked with 10% nonfat dry milk in 0.5% Tris-buffered saline-T (pH 7.6) and incubated with primary antibodies to lumican, decorin, or EGFR for 1 h at a dilution of 1:1000 in 5% nonfat dry milk in 0.5% Tris-buffered saline-T. Secondary horseradish peroxidase-linked donkey or goat antirabbit antibodies (Jackson Immu-noResearch Laboratories, 1 μg/1 μl) were then used, and signals were analyzed by SuperSignal West Pico Chemilu-minescent Substrate (Pierce). Chemiluminescence was pho-tographed before quantitation by video image analysis (Quantity One), and densitometry was analyzed using an MCID M4 system and software (Imaging Research, St. Catherines, Ontario, Canada). The molecular sizes of lumican and decorin ranged from M_r 40,000 to 180,000 and M_r 47,000 to 62,000 in breast tumors as shown previously (10), and all bands were included in the densitometric analysis of each protein.

Statistics and Analysis. Lumican, decorin, and EGFR protein signals were normalized to an appropriate external standard included with the tumor samples on every membrane. The standard was either pooled breast tumor (lumican), pooled normal breast (decorin), or pooled ER-negative breast tumor lysates (EGFR). Normalized lumican and decorin levels were then further adjusted for the proportion of the tissue section occupied by collagenous stroma determined from assessment and scoring of the paraffin section for every case as described previously (10). Associations with clinical-pathological vari-ables were determined by Mann-Whitney or Kruskal-Wallis tests, as appropriate, and correlations were assessed by the Spearman test. Relapse-free survival was defined as the time from initial surgery to the date of clinically documented local or distant disease recurrence or death attributed to breast cancer. Overall survival was defined the time from initial surgery to the date of death attributed to breast cancer. Deaths caused by other known or unknown causes were censored. The association with relapse and survival was assessed by both univariate (Log-rank test and Kaplan-Meier method) and multivariate (Cox regres-sion model) analysis. For univariate survival analysis relative to lumican or decorin expression or lumican:decorin ratio, expres-sion levels $\geq 25^{th}$ percentile for each variable were considered as high levels of expression, because we have observed previ-ously that levels of lumican protein are higher relative to normal tissue in ~75% of tumors. All tests were performed using SAS statistical analysis software.

RESULTS

The clinical-pathological features of this selected cohort of 140 women with invasive breast cancer are described in detail in Table 1. Among these patients, the median age was 69 years, the

median tumor size was 2.5 cm, and the median ER and PR levels were 43 and 31 fmol/mg, respectively. After surgery, 51 patients received postoperative breast radiotherapy. All patients received postoperative adjuvant tamoxifen therapy but no chem-otherapy. The median duration of follow-up for the entire cohort was 54 months. At the time of analysis, recurrences had oc-curred in 11 women (8%), and 16 women (11.4%) had died of disease. Among the remaining 113 censored cases (81%), 95 women were alive and well, 16 women had died of other causes, and 2 women had been lost to follow-up.

Lumican and decorin protein expression was detectable in all 140 cases (Fig. 1). Lumican and decorin core proteins are known to be modified by the addition of glycosaminoglycan side chains and that the degree of modification varies between tissues (17). The molecular sizes of lumican and decorin ranged from M_r 40,000 to 180,000 and M_r 47,000 to 62,000 in breast tumors as shown previously (10), and their relative expression varied over a wide range (mean ^{sd} lumican = 6.07 $^{5.94}$, decorin = 2.35 $^{1.97}$, lumican:decorin ratio = 2.99 $^{1.85}$, measured in arbitrary density units). When low and high levels of expres-sion were considered (using a cutoff point equivalent to the 25th percentile) in relation to tumor characteristics, a lower lumican level was significantly associated with several poor prognostic factors, including low ER and PR, larger size, and increased inflammation, whereas a lower decorin level was only signifi-cantly associated with larger tumor size (Table 2). Neither lumican nor decorin was significantly different between tumor types. Similarly, when expression of either protein was consid-ered as a continuous variable, lumican was significantly lower in tumors associated with poor prognostic factors, including low ER (ER − ve *versus* ER + ve, lumican mean ^{sd} = 3.4 $^{2.5}$, 6.4, $^{6.2}$, $P = 0.0022$), low PR (PR − ve *versus* PR + ve, mean ^{sd} = 5.5 ^{8}, 6.3 $^{4.7}$, $P = 0.013$), large size (≤ 2 cm *versus* >2 cm, mean ^{sd} = 6.8 $^{4.3}$, 5.7 $^{6.6}$, $P = 0.0087$), high inflammation (low *versus* high mean ^{sd} = 6.4 $^{6.3}$, 4 $^{2.3}$, $P = 0.0256$), and also high grade (high *versus* intermediate *versus* low grade, mean ^{sd} = 4.3 $^{2.9}$, 6.3 $^{6.9}$, 6.8 $^{5.5}$, $P = 0.0453$, Kruskal-Wallis) but not EGFR (low *versus* high, mean ^{sd} = 7.5 $^{12.4}$, 4.8 $^{3.5}$, $P = $ ns). Once again, no significant difference was observed in the mean levels of decorin between subgroups of grade, size, inflammation, ER, PR, or EGFR. Although lumican and decorin expression were highly correlated ($r = 0.453$, $P < 0.0001$), analysis of the lumican:decorin ratio as a parameter showed that lower lumi-can:decorin was nevertheless associated ($P < 0.05$) with high grade and ER-negative status.

In the univariate analysis, all established prognostic factors and also higher levels of inflammation were significantly asso-ciated with outcome. Specifically, early recurrence and poor survival were associated with ER − ve ($P < 0.0001$ and 0.0001) or PR − ve ($P < 0.0001$ and 0.0001) status, high EGFR ($P = 0.0125$ and 0.0125), high grade ($P = 0.0176$ and 0.0142), large size ($P = 0.011$ and 0.024), and high inflammation ($P = 0.017$ and 0.0451). When either lumican or decorin expression was considered in relation to outcome (Fig. 2), significant associa-tions were found with low levels of expression, early recurrence (lumican $P = 0.0013$, decorin $P = 0.026$), and poor survival (lumican $P = 0.001$, decorin $P = 0.0076$). This association remained unchanged when the analysis was limited to only invasive ductal or lobular carcinomas (overall survival $n = 127$,
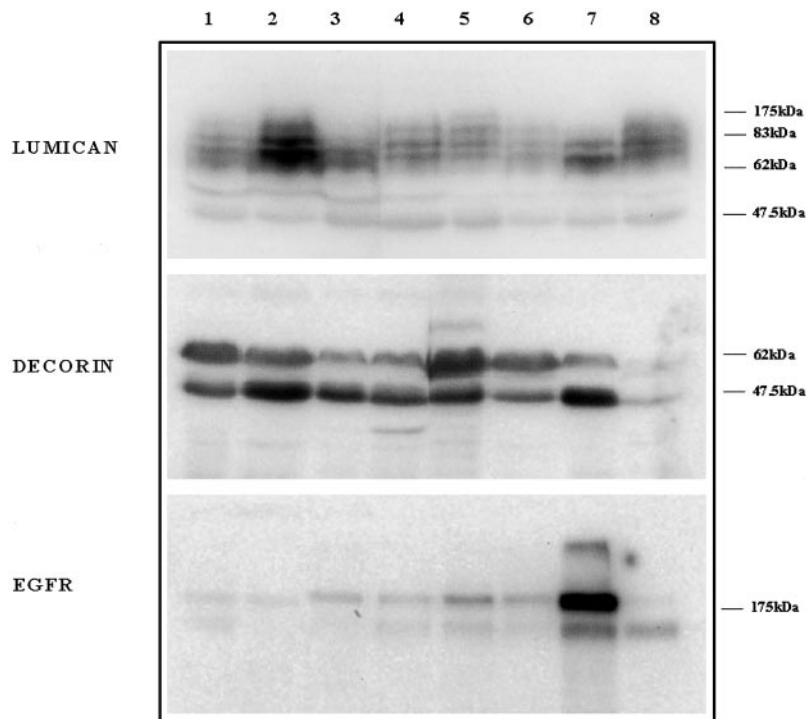
Fig. 1 Western blot analysis of lumican, decorin, and EGFR protein expression in breast tumors. *Lanes* correspond to representative ER-positive tumors (*1–6, 8*) and an ER-negative tumor (*Lane 7*).

Table 2 Univariate analysis of the relationship between lumican and decorin expression and other prognostic factors[a]

		Lumican			Decorin			Lumican/decorin		
		Low	High	P	Low	High	P	Low	High	P
ER	−ve	9	6	0.0024*	3	12	ns	8	7	0.0073
	+ve	26	99		32	93		27	98	
PR	−ve	17	27	0.0116	14	30	ns	14	30	ns
	+ve	18	78		21	75		21	75	
EGFR	Low	12	23	ns	13	22	ns	9	26	ns
	High	12	23		10	25		9	26	
Grade	Low	10	37	ns	16	31	ns	10	37	ns
	Int	14	53		13	54		15	52	
	High	11	15		6	20		10	16	
Size	≤2	7	40	0.0496	7	40	0.0496	6	41	0.0175
	>2	28	65		28	65		29	64	
INFL[b]	Low	25	96	0.0077*	30	91	ns	27	94	ns
	High	10	9		5	14		8	11	

[a] All associations are based on χ^2 test or where indicated * Fisher's exact test.
[b] INFL, inflammation.

lumican $P = 0.0027$, decorin $P = 0.0088$) or when the analysis was limited to only ER + ve cases (overall survival $n = 125$, lumican $P = 0.0086$, decorin $P = 0.014$). However, although a lower lumican:decorin ratio showed a trend toward worse survival, this was not significant. The relationship between lumican, decorin, and EGFR expression measured by the same method, and outcome, was evaluated in those cases where sufficient frozen tissue was available for additional Western blot study; however, the characteristics of this subcohort ($n = 70$) were not significantly different from the entire cohort (Table 1). In tumors with high EGFR expression ($n = 35/70$), the level of

lumican expression was not significantly associated with outcome; however, low levels of decorin were associated with a significantly higher risk of recurrence ($P = 0.0102$) and survival ($P = 0.0004$).

Multivariate analysis using the Cox proportional hazards model was performed. When either forward or backward model selection procedures were used and considering ER and PR status, tumor size, grade, inflammatory response, and patient age together with either lumican or decorin status, only decorin, ER, and PR emerged as independent predictors of recurrence and survival (Table 3).

Fig. 2 Kaplan-Meier graphs for "time to progression" and "overall survival" with respect to expression of SLRPs. Lumican progression (*top left*), low lumican $n = 35$, events = 12; high lumican $n = 105$, events = 15. Lumican survival (*bottom left*), low lumican $n = 35$, events = 9; high lumican $n = 105$, events = 7. Decorin progression (*top right*), low decorin $n = 35$, events = 11; high decorin $n = 105$, events = 16. Decorin survival (*bottom right*), low decorin $n = 35$, events = 8, high decorin $n = 105$, events = 8.
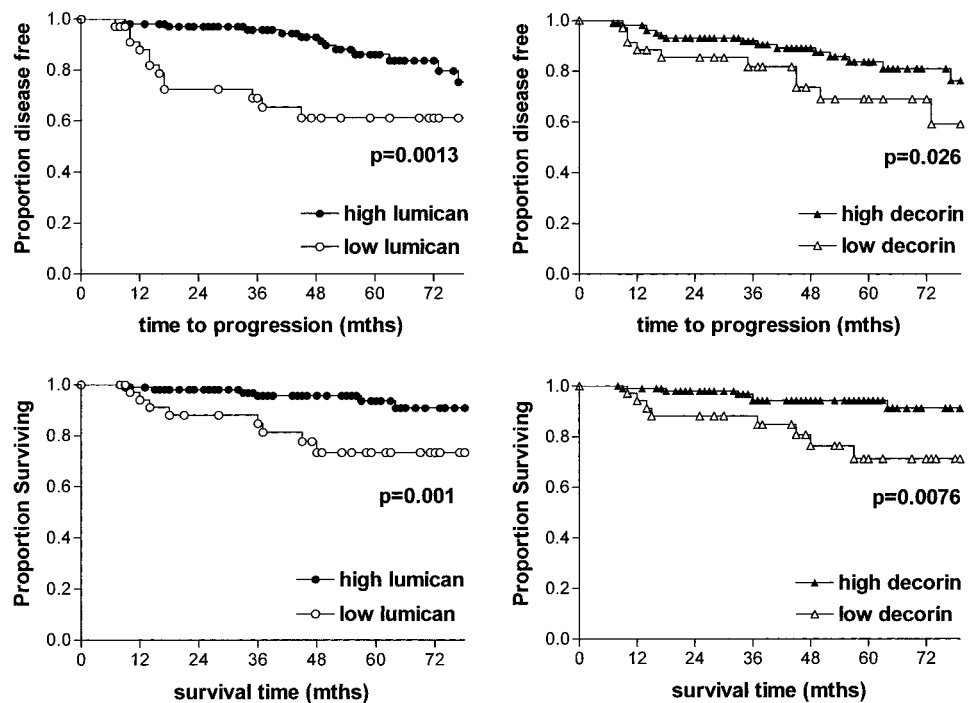
Table 3 Cox multivariate analysis for survival

	Odds ratio	95% CI[a]	Significance
Recurrence-free survival			
ER	6.1	2.59–14.36	<0.0001
PR	4.03	1.69–9.58	0.0016
Decorin	2.25	1.01–5.0	0.047
Overall survival			
PR	12.28	2.64–57.0	0.0014
ER	3.86	1.31–11.36	0.0142
Decorin	3.39	1.21–9.55	0.0206

[a] CI, confidence interval.

DISCUSSION

We have shown that low levels of both lumican and decorin may be associated with early recurrence and poor survival within node-negative invasive breast cancers treated by endocrine therapy. In the case of decorin, the association with outcome is also maintained within the subset of these tumors that show higher EGFR expression and is independent of other clinical prognostic markers. We chose to examine the relationship of these SLRPs with outcome specifically in early stage carcinomas, where stromal integrity might be expected to be important in limiting invasion and metastasis, and in a cohort that had been uniformly treated by endocrine therapy, because in this clinical subset, there is both *in vitro* and *in vivo* data to indicate that the activity of the EGFR growth factor pathway, which may be influenced by decorin levels, is functionally pertinent to outcome (18, 19).

Breast carcinoma develops out of alterations in the expression of multiple genes and cellular pathways. Many of these genes reside within and directly affect the breast epithelial cell. However, alterations can also occur within the stromal compartment and can influence tumor cell behavior through either paracrine growth factor pathways or effects on the composition and architecture of the ECM (20). The influence of the ECM can be exerted both by biochemical and mechanical effects (20, 21). The former works through changes in the transfer, storage, and activation of growth factors, as well as their delivery and accessibility to the relevant receptors on epithelial cells. The latter, *e.g.,* works through the engagement of adhesion receptors that elicit intracellular signaling responses and that intersect and synergize with growth factor pathways (22–24).

The SLRPs are important components of the ECM. Decorin is the best studied of these genes and is known to be capable of influencing stromal structure through direct effects on collagen fibril growth and assembly both *in vitro* and *in vivo,* as well as stromal matrix production through binding to and inactivation of TGF-β. Decorin can also affect epithelial tumor cell growth (25, 26), through indirect effects on the availability of growth factors or directly through activation of the EGFR (13, 27). The interaction with EGFRs causes subunit dimerization, activation of the mitogen-activated protein kinase pathway, and induction of the p21[waf1] cell cycle inhibitor (27, 28). Finally, decorin can influence lymphoma tumorigenesis in mouse models (14). In contrast, less is known about lumican and other SLRPs (29). Although only decorin appears to interact with the EGFR, all of the SLRPs, including lumican (30), have been shown to interact with TGF-β with comparable affinity to decorin *in vitro*. However, their effects on TGF-β *in vivo* may be different. All are important in the regulation of collagen fibril assembly (8), and targeted mutation of all of the four major SLRPs in mice has yielded predictable phenotypes related to the connective tissues. Homozygous deletion of the decorin, lumi-

can, and fibromodulin genes causes alteration of the ECM in skin and/or tendon associated with disorganized collagen fibers and increased or irregular fibril size and interfibrillar spacing, as viewed by light and electron microscopy (31–34). Ocular opacity was also found in lumican-null mice, whereas the biglycan-null mice demonstrate an osteoporosis-like phenotype (32, 34). Thus, the data from knockout mouse experiments indicate that lumican, decorin, and the other SLRPs are very important proteins in the regulation of collagen fibril assembly and structure in different normal tissues, with a potentially important role in the modulation of ECM signaling (8, 30).

It is therefore reasonable to consider the role of the SLRPs in tumor growth and invasion, and both positive and negative roles have been proposed for decorin and biglycan (11, 35). A role for altered lumican expression has not been considered previously; however, the potential for similar effects on stromal integrity and the similar capacity to bind TGF-β might suggest a similar effect. Our finding here that low levels of decorin and also lumican are associated with more aggressive disease is consistent with the view that reduced decorin or lumican expression may facilitate tumorigenesis, invasion, and/or growth.

One explanation for this observation may lie in the influence of SLRP expression on the structural properties of the stromal reaction and fibrosis at the invasive edge of breast tumors that may serve to limit invasion (36). Because both decorin and lumican are important in maintaining normal collagen structure, their reduced expression may weaken the matrix and reduce the effectiveness of this response as a physical barrier to tumor spread (36).

Another explanation may be that lower expression of SLRPs, and in particular decorin, reduces the capacity of the newly formed stroma to bind and sequester TGF-β and so may modulate the bioactivity of this growth factor. One concept is that higher TGF-β inhibits tumor growth by stimulation of synthesis of ECM and fibrosis and also by direct inhibition of tumor cell proliferation. However, this has been challenged by contradictory evidence, and another current view is that the net effect of TGF-β may change with tumorigenesis and actually be inhibitory in early stages and stimulatory at later invasive stages of epithelial tumors (37–39). A potential role for increased TGF-β in mediating resistance to tamoxifen therapy has also emerged from *in vitro* and *in vivo* data but has yet to be resolved (40). Recent studies suggest that higher decorin levels frequently lead to a reduction in TGF-β responsiveness in stromal cells (41–43), although contradictory effects occur in some systems. The relative tissue localization may also influence the cumulative effect (41).

A third explanation lies in the specific effect of decorin on signaling mediated by EGFR and other Erb-B family receptors (27). Alteration of EGF response has been identified as an important step in breast preneoplasia (44) and may influence growth, adhesion, and invasion (45, 46). In advanced invasive disease, the EGFR has also been implicated as a marker of poor relapse-free survival and resistance to endocrine therapy (47). Our findings here, even though limited to a small sub-cohort of tumors, suggest that low decorin levels in the ECM may indeed exert a specific influence on tumors with high levels of EGFR and may affect outcome after treatment by endocrine therapy.

Our previous studies identified lumican and decorin as the most highly expressed members of the SLRP gene family in breast tumors, and in a small set of invasive breast cancers, higher levels of mRNA expression were apparently associated with poor prognostic markers (9). However, we were unable to confirm the associations with prognostic markers at the protein level in a subsequent study (10). We also found previously that these two SLRPs are differentially regulated at the mRNA level in early stages of tumorigenesis, such that lumican mRNA is consistently elevated, whereas decorin expression is reduced in neoplastic stroma as compared with normal tissue adjacent to the invasive margin. Nevertheless, within different tumors, the relative levels of expression of these SLRPs were found to be highly correlated. The current study focusing only on invasive tumors confirms that the expression of lumican and decorin is correlated. The finding here of associations between low lumican levels and some of the same poor prognostic parameters is surprising but may be attributable to several factors. These include the larger cohort size, the restriction of the current series to node-negative tumors, and also the different measurement of lumican expression, given the observation that discordance can exist between lumican mRNA and protein expression within both neoplastic and normal tissues (10).

In summary, we have shown that low lumican and decorin expression have prognostic significance for a shorter time to relapse and a worse survival among women with node-negative breast cancer treated by endocrine therapy. Given the indication that this relationship persists within the subset of patients with high EGFR expression and the capability of decorin to modulate EGFR and HER-2/Erb-B2 signaling, it will be important to examine whether SLRP expression modifies the prognostic significance of EGFR and HER-2/Erb-B2 in breast cancer or influences the outcome to Herceptin therapy. There are also potential advantages for considering the targeting and manipulation of components of the "stable" stroma rather than the "adaptable" epithelial tumor cells (48), and manipulation of SLRP expression *in vivo* to possibly influence stromal architecture and growth factor pathways has already been demonstrated. Confirmation of the prognostic significance of SLRP gene expression in larger studies is clearly warranted.

REFERENCES

1. Morrow, M. Identification of the woman at risk for breast cancer: problem solved? Recent Results Cancer Res., *151:* 85–95, 1999.

2. Silverstein, M. J., Lagios, M. D., Craig, P. H., Waisman, J. R., Lewinsky, B. S., Colburn, W. J., and Poller, D. N. A prognostic index for ductal carcinoma in situ of the breast. Cancer (Phila.), *77:* 2267–2274, 1996.

3. Liotta, L. A. Tumor invasion and metastases: role of the basement membrane. Warner-Lambert Parke-Davis Award lecture. Am. J. Pathol., *117:* 339–348, 1984.

4. Lochter, A., and Bissell, M. J. Involvement of extracellular matrix constituents in breast cancer. Semin. Cancer Biol., *6:* 165–173, 1995.

5. Smith-Mungo, L. I., and Kagan, H. M. Lysyl oxidase: properties, regulation and multiple functions in biology. Matrix Biol., *16:* 387–398, 1998.

6. Kivirikko, K. I. Collagens and their abnormalities in a wide spectrum of diseases. Ann. Med., *25:* 113–126, 1993.

7. Kyriakides, T. R., Zhu, Y. H., Smith, L. T., Bain, S. D., Yang, Z., Lin, M. T., Danielson, K. G., Iozzo, R. V., LaMarca, M., McKinney, C. E., Ginns, E. I., and Bornstein, P. Mice that lack thrombospondin 2 display connective tissue abnormalities that are associated with disordered collagen fibrillogenesis, an increased vascular density, and a bleeding diathesis. J. Cell Biol., *140:* 419–430, 1998.

8. Iozzo, R. V. The family of the small leucine-rich proteoglycans: key regulators of matrix assembly and cellular growth. Crit. Rev. Biochem. Mol. Biol., *32:* 141–174, 1997.

9. Leygue, E., Snell, L., Dotzlaw, H., Hole, K., Hiller-Hitchcock, T., Roughley, P. J., Watson, P. H., and Murphy, L. C. Expression of lumican in human breast carcinoma. Cancer Res., *58:* 1348–1352, 1998.

10. Leygue, E., Snell, L., Dotzlaw, H., Troup, S., Hiller-Hitchcock, T., Murphy, L. C., Roughley, P. J., and Watson, P. H. Lumican and decorin are differentially expressed in human breast carcinoma. J. Pathol., *192:* 313–320, 2000.

11. Iozzo, R. V., and Cohen, I. Altered proteoglycan gene expression and the tumor stroma. Experientia, *49:* 447–455, 1993.

12. Iozzo, R. V. Tumor stroma as a regulator of neoplastic behavior. Agonistic and antagonistic elements embedded in the same connective tissue. Lab. Investig., *73:* 157–160, 1995.

13. Moscatello, D. K., Santra, M., Mann, D. M., McQuillan, D. J., Wong, A. J., and Iozzo, R. V. Decorin suppresses tumor cell growth by activating the epidermal growth factor receptor. J. Clin. Investig., *101:* 406–412, 1998.

14. Iozzo, R. V., Chakrani, F., Perrotti, D., McQuillan, D. J., Skorski, T., Calabretta, B., and Eichstetter, I. Cooperative action of germ-line mutations in decorin and p53 accelerates lymphoma tumorigenesis. Proc. Natl. Acad. Sci. USA, *96:* 3092–3097, 1999.

15. Watson, P. H., Snell, L., and Parisien, M. The NCIC-Manitoba Breast Tumor Bank: a resource for applied cancer research. CMAJ, *155:* 281–283, 1996.

16. Cs-Szabo, G., Melching, L. I., Roughley, P. J., and Glant, T. T. Changes in messenger RNA and protein levels of proteoglycans and link protein in human osteoarthritic cartilage samples. Arthritis Rheum., *40:* 1037–1045, 1997.

17. Grover, J., Chen, X. N., Korenberg, J. R., and Roughley, P. J. The human lumican gene. Organization, chromosomal location, and expression in articular cartilage. J. Biol. Chem., *270:* 21942–21949, 1995.

18. Fox, S. B., Smith, K., Hollyer, J., Greenall, M., Hastrich, D., and Harris, A. L. The epidermal growth factor receptor as a prognostic marker: results of 370 patients and review of 3009 patients. Breast Cancer Res. Treat., *29:* 41–49, 1994.

19. Harris, A. L., Nicholson, S., Sainsbury, J. R., Farndon, J., and Wright, C. Epidermal growth factor receptors in breast cancer: association with early relapse and death, poor response to hormones and interactions with neu. J. Steroid Biochem., *34:* 123–131, 1989.

20. Bissell, M. J., Weaver, V. M., Lelievre, S. A., Wang, F., Petersen, O. W., and Schmeichel, K. L. Tissue structure, nuclear organization, and gene expression in normal and malignant breast. Cancer Res., *59:* 1757–1763s, 1999.

21. Roskelley, C. D., and Bissell, M. J. Dynamic reciprocity revisited: a continuous, bidirectional flow of information between cells and the extracellular matrix regulates mammary epithelial cell function. Biochem. Cell Biol., *73:* 391–397, 1995.

22. Gardner, H., Broberg, A., Pozzi, A., Laato, M., and Heino, J. Absence of integrin alpha1beta1 in the mouse causes loss of feedback regulation of collagen synthesis in normal and wounded dermis. J. Cell Sci., *112:* 263–272, 1999.

23. Schlessinger, J. Direct binding and activation of receptor tyrosine kinases by collagen. Cell, *91:* 869–872, 1997.

24. Roskelley, C. D., Desprez, P. Y., and Bissell, M. J. Extracellular matrix-dependent tissue-specific gene expression in mammary epithelial cells requires both physical and biochemical signal transduction. Proc. Natl. Acad. Sci. USA, *91:* 12378–12382, 1994.

25. Santra, M., Skorski, T., Calabretta, B., Lattime, E. C., and Iozzo, R. V. De novo decorin gene expression suppresses the malignant phe-

notype in human colon cancer cells. Proc. Natl. Acad. Sci. USA, *92:* 7016–7020, 1995.

26. Santra, M., Mann, D. M., Mercer, E. W., Skorski, T., Calabretta, B., and Iozzo, R. V. Ectopic expression of decorin protein core causes a generalized growth suppression in neoplastic cells of various histogenetic origin and requires endogenous p21, an inhibitor of cyclin-dependent kinases. J. Clin. Investig., *100:* 149–157, 1997.

27. Iozzo, R. V., Moscatello, D. K., McQuillan, D. J., and Eichstetter, I. Decorin is a biological ligand for the epidermal growth factor receptor. J. Biol. Chem., *274:* 4489–4492, 1999.

28. De Luca, A., Santra, M., Baldi, A., Giordano, A., and Iozzo, R. V. Decorin-induced growth suppression is associated with up-regulation of p21, an inhibitor of cyclin-dependent kinases. J. Biol. Chem., *271:* 18961–18965, 1996.

29. Hildebrand, A., Romaris, M., Rasmussen, L. M., Heinegard, D., Twardzik, D. R., Border, W. A., and Ruoslahti, E. Interaction of the small interstitial proteoglycans biglycan, decorin and fibromodulin with transforming growth factor beta. Biochem. J., *302:* 527–534, 1994.

30. Iozzo, R. V. The biology of the small leucine-rich proteoglycans. Functional network of interactive proteins. J. Biol. Chem., *274:* 18843–18846, 1999.

31. Danielson, K. G., Baribault, H., Holmes, D. F., Graham, H., Kadler, K. E., and Iozzo, R. V. Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. J. Cell Biol., *136:* 729–743, 1997.

32. Chakravarti, S., Magnuson, T., Lass, J. H., Jepsen, K. J., LaMantia, C., and Carroll, H. Lumican regulates collagen fibril assembly: skin fragility and corneal opacity in the absence of lumican. J. Cell Biol., *141:* 1277–1286, 1998.

33. Svensson, L., Aszodi, A., Reinholt, F. P., Fassler, R., Heinegard, D., and Oldberg, A. Fibromodulin-null mice have abnormal collagen fibrils, tissue organization, and altered lumican deposition in tendon. J. Biol. Chem., *274:* 9636–9647, 1999.

34. Xu, T., Bianco, P., Fisher, L. W., Longenecker, G., Smith, E., Goldstein, S., Bonadio, J., Boskey, A., Heegaard, A. M., Sommer, B., Satomura, K., Dominguez, P., Zhao, C., Kulkarni, A. B., Robey, P. G., and Young, M. F. Targeted disruption of the biglycan gene leads to an osteoporosis-like phenotype in mice. Nat. Genet., *20:* 78–82, 1998.

35. Weber, C. K., Sommer, G., Michl, P., Fensterer, H., Weimer, M., Gansauge, F., Leder, G., Adler, G., and Gress, T. M. Biglycan is overexpressed in pancreatic cancer and induces G1-arrest in pancreatic cancer cell lines. Gastroenterology, *121:* 657–667, 2001.

36. Peyrol, S., Raccurt, M., Gerard, F., Gleyzal, C., Grimaud, J. A., and Sommer, P. Lysyl oxidase gene expression in the stromal reaction to in situ and invasive ductal breast carcinoma. Am. J. Pathol., *150:* 497–507, 1997.

37. Akhurst, R. J., and Balmain, A. Genetic events and the role of TGF beta in epithelial tumour progression. J. Pathol., *187:* 82–90, 1999.

38. Reiss, M., and Barcellos-Hoff, M. H. Transforming growth factor-beta in breast cancer: a working hypothesis. Breast Cancer Res. Treat., *45:* 81–95, 1997.

39. Arteaga, C. L., Dugger, T. C., and Hurd, S. D. The multifunctional role of transforming growth factor (TGF)-beta s on mammary epithelial cell biology. Breast Cancer Res. Treat., *38:* 49–56, 1996.

40. Arteaga, C. L., Koli, K. M., Dugger, T. C., and Clarke, R. Reversal of tamoxifen resistance of human breast carcinomas in vivo by neutralizing antibodies to transforming growth factor-beta. J. Natl. Cancer Inst., *91:* 46–53, 1999.

41. Kolb, M., Margetts, P. J., Sime, P. J., and Gauldie, J. Proteoglycans decorin and biglycan differentially modulate TGF-beta-mediated fibrotic responses in the lung. Am. J. Physiol. Lung Cell Mol. Physiol., *280:* L1327–L1334, 2001.

42. Fischer, J. W., Kinsella, M. G., Levkau, B., Clowes, A. W., and Wight, T. N. Retroviral overexpression of decorin differentially affects the response of arterial smooth muscle cells to growth factors. Arterioscler. Thromb. Vasc. Biol., *21:* 777–784, 2001.

43. Markmann, A., Hausser, H., Schonherr, E., and Kresse, H. Influence of decorin expression on transforming growth factor-beta-mediated collagen gel retraction and biglycan induction. Matrix Biol., *19:* 631–636, 2000.

44. Medina, D., Kittrell, F. S., Oborn, C. J., and Schwartz, M. Growth factor dependency and gene expression in preneoplastic mouse mammary epithelial cells. Cancer Res., *53:* 668–674, 1993.

45. Genersch, E., Schneider, D. W., Sauer, G., Khazaie, K., Schuppan, D., and Lichtner, R. B. Prevention of EGF-modulated adhesion of tumor cells to matrix proteins by specific EGF receptor inhibition. Int. J. Cancer, *75:* 205–209, 1998.

46. Verbeek, B. S., Adriaansen-Slot, S. S., Vroom, T. M., Beckers, T., and Rijksen, G. Overexpression of EGFR and c-erbB2 causes enhanced cell migration in human breast cancer cells and NIH3T3 fibroblasts. FEBS Lett., *425:* 145–150, 1998.

47. Nicholson, S., Wright, C., Sainsbury, J. R., Halcrow, P., Kelly, P., Angus, B., Farndon, J. R., and Harris, A. L. Epidermal growth factor receptor (EGFr) as a marker for poor prognosis in node-negative breast cancer patients: neu and tamoxifen failure. J. Steroid Biochem. Mol. Biol., *37:* 811–814, 1990.

48. Kerbel, R. S. New targets, drugs, and approaches for the treatment of cancer: an overview. Cancer Metastisis Rev., *17:* 145–147, 1998.

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