Evaluating the Expression and Prognostic Value of TRAIL-R1 and TRAIL-R2 in Breast Cancer

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Abstract

Purpose: The cell surface receptors tumor necrosis factor–related apoptosis-inducing ligand receptor 1 (TRAIL-R1) and TRAIL-R2 transmit apoptotic signals, and agents that activate these receptors are in clinical development. We sought to determine the expression and prognostic value of TRAIL-R1 and TRAIL-R2 in early-stage breast cancer.

Experimental Design: Tissue microarrays containing specimens from 655 breast cancer patients with 20-year follow-up were employed and evaluated with our automated quantitative analysis (AQUA) system. The system uses cytokeratin to define pixels as breast cancer (tumor mask) within the array spot, and measures intensity of TRAIL receptor expression using Cy5 conjugated antibodies within the mask. AQUA scores were correlated with clinical and pathologic variables. TRAIL-R1 and TRAIL-R2 expression were similarly studied on 95 unmatched normal breast specimens.

Results: TRAIL-R1 expression was not associated with survival. High TRAIL-R2 expression strongly correlated with decreased survival ($P = 0.0005$). On multivariate analysis, high TRAIL-R2 expression remained an independent prognostic marker, as did nodal status and tumor size. High TRAIL-R2 expression correlated strongly with lymph node involvement ($P = 0.0003$). TRAIL-R2 expression was stronger in malignant specimens than in normal breast epithelium ($P < 0.0001$).

Conclusions: High TRAIL-R2 expression was independently associated with decreased survival in breast cancer. The biological basis and the sensitivity of high TRAIL-R2 expressing cells to TRAIL agonists and/or chemotherapy are subject to further investigation. Evaluation of TRAIL-R2 expression in early-stage breast cancer may identify a subset of patients requiring more aggressive or pathway-targeted adjuvant treatment. Clinical trials involving TRAIL-R2 agonists should stratify patients based on TRAIL-R2 expression.

The tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) cell surface receptors 1 and 2 (TRAIL-R1 and TRAIL-R2, also known as death receptor 4 and death receptor 5) belong to the tumor necrosis factor receptor gene superfamily, and are capable of transmitting signals that cause apoptosis. Common features of all death receptors include a cysteine-rich, extracellular domain and a homologous cytoplasmic sequence termed the "death domain," which the receptors use to engage the apoptotic machinery of the cell (1). Once the death ligand binds to its receptor, proteins of the death-inducing signaling complex are recruited to the death domain and the apoptosis signal is activated. Molecules other than the death ligands may bind to the death-inducing signaling complex and antagonize the death signal, allowing the cell to survive (2).

Sensitivity of cancer cells to TRAIL-induced apoptosis has been shown by numerous researchers. Therefore, the TRAIL ligand has been the subject of investigation as a potential anticancer therapeutic agent. It has shown antitumor activity in cancer cells (3, 4), with limited toxicity to normal cells in mouse models (4). The combination of chemotherapeutic drugs and TRAIL ligand produced synergistic antitumor effects against various malignancies *in vitro* and *in vivo*, including breast cancer (5–10). Recently, clinical trials using TRAIL ligand were initiated (http://www.gene.com).

In addition to TRAIL-R1 and TRAIL-R2, TRAIL interacts with three other receptors, TRAIL-R3, TRAIL-R4, and osteoprotegrin. These latter receptors lack the intracellular death domains essential for the transmission of the apoptotic signal, and serve as decoy receptors. Due to the apparent competition between proapoptotic TRAIL receptors and the decoy receptors, molecules that selectively activate the proapoptotic receptors may have therapeutic advantages compared with the TRAIL ligand.

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Activating monoclonal antibodies to TRAIL-R1 and TRAIL-R2 have shown single agent antitumor activity (11, 12). The antibody against TRAIL-R2 showed synergistic activity in combination with Adriamycin in a breast cancer model (12). Agonistic monoclonal antibodies to TRAIL-R1 and TRAIL-R2 are currently under development (http://www.hgsi.com).

Due to the development of targeted therapies for TRAIL-R1 and TRAIL-R2 activation, we sought to determine the expression of these two targets in tumors from a large cohort of breast cancer patients, and to associate expression with clinical outcome. As has been the case with some other targeted therapies, such as trastuzumab for breast cancer, it is possible that response to these drugs might be associated with the level of target expression in the tumors (13). Furthermore, markers that have both prognostic and therapeutic value, such as HER2/neu and hormone receptors, have significantly affected our ability to select therapeutic regimens in early-stage breast cancer. To obtain more accurate, objective measures of expression, we used our newly developed method of automated quantitative analysis (AQUA) of tissue microarrays. This method has been validated, has proven to be more accurate than pathologist-based scoring of brown stain (14–16), and has been used in a number of prior studies (15–19).

Materials and Methods

Tissue microarray construction. The breast cancer tissue microarrays were constructed as previously described (20). A total of 331 node-negative and 324 node-positive breast cancer cores, each measuring 0.6 mm in diameter, were spaced 0.8 mm apart on two glass slides. The cohort was constructed from paraffin-embedded, formalin-fixed tissue blocks obtained from the Yale University Department of Pathology Archives. Specimens and clinical information were collected under the guidelines and approval of a Yale University Institutional Review Board. Estrogen receptor staining was positive in 52%, progesterone receptor in 46%, and HER2/neu in 14%. Nuclear grade 3 (on a 1-3 scale) was seen in 28% of the specimens, and 59% were larger than 2 cm. The histologic subtypes included 72% invasive ductal carcinoma, 1% lobular carcinoma, and 14% had mixed or other histology. The specimens were resected between 1962 and 1980, with a follow-up range between 4 months and 53 years, and a mean follow-up time of 12.6 years. Age at diagnosis ranged from 24 to 88 years (mean age, 58 years). Complete treatment history was not available for the entire cohort. Most patients were treated with local irradiation. None of the node-negative patients were given adjuvant systemic therapy. A minority of the node-positive patients (~15%) received chemotherapy, and ~27% received tamoxifen (after 1978). The time between tumor resection and tissue fixation was not available. A pathologist reviewed slides from all of the blocks to select representative areas of invasive tumor to be cored. The cores were placed on the tissue microarray using a Tissue Microarrayer (Beecher Instruments, Silver Spring, MD). The tissue microarrays were then cut to 0.5 μm sections and placed on glass slides using an adhesive tape-transfer system (Instrumedics, Inc., Hackensack, NJ) with UV cross-linking. Similarly, a tissue microarray was made containing cores from 95 unmatched benign breast specimens.

Immunohistochemistry. Staining was done for automated analysis of breast cancer specimens as previously described (14). Briefly, slides were deparaffinized in xylene and transferred through two changes of 100% ethanol. For antigen retrieval, the slides were boiled in a pressure cooker containing 6.5 mmol/L sodium citrate (pH 6.0). Endogenous peroxidase activity was blocked in a mixture of methanol and 2.5% hydrogen peroxide for 30 minutes at room temperature. To reduce nonspecific background staining, slides were incubated at room temperature for 30 minutes in 0.3% bovine serum albumin/1× TBS. Slides were incubated at 4°C overnight in a humidity tray with the primary antibodies [rabbit polyclonal anti–TRAIL-R2 immunoglobulin G at 1:350 (Oncogene Research, San Diego, CA) and mouse monoclonal anti–TRAIL-R1 immunoglobulin G at 1:80 (R&D Systems, Inc., Minneapolis, MN)]. To create a tumor mask, slides were simultaneously incubated overnight with a primary anticytokeratin antibody (rabbit anti-human cytokeratin for TRAIL-R1 and mouse anti-human cytokeratin for TRAIL-R2) at a dilution of 1:200. Slides were rinsed thrice in 1× TBS/0.05% Tween 20. For TRAIL-R2, slides were incubated for 1 hour at room temperature with 4,6-diamidino-2-phenylindole at a dilution of 1:100 to identify the nuclei, with goat anti-rabbit horseradish peroxidase (Envision; DAKO Corp., Carpinteria, CA) to identify the target, and with goat anti-mouse immunoglobulin G conjugated to Alexa 488 (Molecular Probes, Inc. Eugene, OR) at a dilution of 1:100 to identify the mask. The same technique was used to assess TRAIL-R1 expression, except that goat anti-mouse horseradish peroxidase and goat anti-rabbit immunoglobulin G conjugated to Alexa 488 were used. The slides were washed again as above and incubated for 10 minutes with Cy5 directly conjugated to tyramide (Perkin-Elmer, Boston, MA) at a dilution of 1:50 for primary antibody identification. The slides were rinsed again and coverslips were mounted.

Automated image acquisition. Images were acquired using automated quantitative analysis (AQUA), as previously described (14, 15). Briefly, areas of tumor were distinguished from stroma by creating a mask with the cytokeratin signal tagged with Alexa 488. Coalescence of cytokeratin at the cell surface was used to identify the membrane/cytoplasm compartment within the tumor mask, whereas 4,6-diamidino-2-phenylindole was used to identify the nuclear compartment within the tumor mask. The target markers, TRAIL-R1 and TRAIL-R2, were visualized with Cy5 (red). Cy5 was used because its emission peak is outside the color spectrum of tissue autofluorescence (14). Multiple monochromatic, high-resolution (1,024 × 1,024 pixel, 0.5 μm) gray-scale images were obtained for each histospot, using the 10× objective of an Olympus AX-51 epifluorescence microscope (Olympus, Melville, NY) with an automated microscope stage and digital image acquisition driven by custom program and macro-based interfaces with IPLabs software (Scanalytics, Inc., Fairfax, VA).

Algorithmic image analysis. Images were analyzed using algorithms that have been previously extensively described (14). Two images (one in-focus and one out-of-focus) were taken of the compartment specific tags and the target marker. A percentage of the out-of-focus image was subtracted from the in-focus image for each pixel, representing the signal to noise ratio of the image. An algorithm described as Rapid Exponential Subtraction Algorithm was used to subtract the out-of-focus information in a uniform fashion for the entire microarray. Subsequently, the Pixel Locale Assignment for Compartmentalization of Expression algorithm was used to assign each pixel in the image to a specific subcellular compartment and the signal in each location is calculated. Pixels that cannot be accurately assigned to a compartment were discarded. The data were saved and subsequently expressed as the average signal intensity per unit of compartment area. For the nuclear and membrane/cytoplasmic compartments, the image was measured on a scale of 0 to 255, and expressed as target signal intensity relative to the compartment area.

Statistical analysis. The StatView and JMP5 (SAS Institute, Inc., Cary, NC) software packages were used for data analyses. Continuous AQUA scores of target expression were divided into quartiles and associations with clinical and pathologic variables were completed using the χ^2 test. The prognostic significance of the variables was assessed for predictive value using the Cox proportional hazards model with overall survival as an end point. Survival curves were generated using the Kaplan-Meier method, with significance evaluated using the Mantel-Cox log-rank test. Comparison of expression in malignant and benign specimens was done with t tests.

Results

To account for intratumor heterogeneity, two separate sets of slides, each containing a core from a different area of the tumor for each patient, were used to evaluate the expression of each marker. Both receptors did not have significant amounts of nuclear staining, and only the membranous/cytoplasmic compartments were analyzed. Using the Pearson correlation test, we found that the scores from matching spots on the two arrays were highly correlated ($P < 0.0001$) for both TRAIL-R1 and TRAIL-R2 expression. AQUA scores ranged from 7.11 to 197.96 for TRAIL-R1, with a median score of 31.20, and from 19.99 to 157.41 for TRAIL-R2, with a median score of 73.88. Examples of strong and weak TRAIL-R2 staining are shown in Fig. 1.

For each of the two markers, the AQUA scores from both sets of slides were combined to give a single data set. Of the 672 patient tumor histospots on each slide, 387 were interpretable from both cores for TRAIL-R1 and 186 were interpretable for one core. Tumor spots were deemed uninterpretable if they had insufficient tumor cells, loss of tissue in the spot, or an abundance of necrotic tissue. For patients who had two interpretable histospots, a composite score was formed by taking the average of the two scores. For patients with only one interpretable core, the single score was used. The combined data set for TRAIL-R1 had scores for 573 patients. For TRAIL-R2, 319 patients had two scores and 235 had one score, yielding a data set with scores for 554 patients.

Using the Cox univariate survival analysis of raw AQUA scores, we found that TRAIL-R1 expression was not associated with breast cancer specific survival ($P = 0.12$). On the other hand, high TRAIL-R2 expression was strongly correlated with decreased survival in the entire cohort ($P = 0.0005$; Table 1). In breast cancer, it is often useful to examine patient cohorts split by nodal status, as this reflects the standard clinical approach to patients. In the node-negative subset of patients, there was an association between strong TRAIL-R1 and TRAIL-R2 expression and decreased survival ($P = 0.043$ and $P = 0.0205$, respectively), but no significant association was found within the node-positive subset of patients (Table 1).

Continuous AQUA scores were then divided into quartiles, reflecting the use of routine statistical divisions in the absence of an underlying justification for division of expression levels. Kaplan-Meier survival curves were generated for TRAIL-R1 and TRAIL-R2, as shown in Fig. 2A and B. Similarly, we generated Kaplan-Meier survival curves for node-negative patients, as shown in Fig. 2C and D. The log-rank analysis for TRAIL-R1 did not reveal a statistically significant association with survival in the entire cohort ($P = 0.5025$) or in the node-negative group (log-rank $P = 0.2358$), whereas the log-rank analysis for quartiles of TRAIL-R2 expression revealed a significant association with survival in the entire cohort ($P = 0.006$) and among node-negative patients ($P = 0.0136$). Therefore, the rest of the analyses focus on TRAIL-R2 only.

Figure 2B and D shows clear convergence of the second, third, and fourth quartiles of AQUA scores to define the first quartile as "low TRAIL-R2 expressers" and of the second, third, and fourth quartiles as "high TRAIL-R2 expressers." Using the Cox proportional hazards model, we did multivariate analyses to

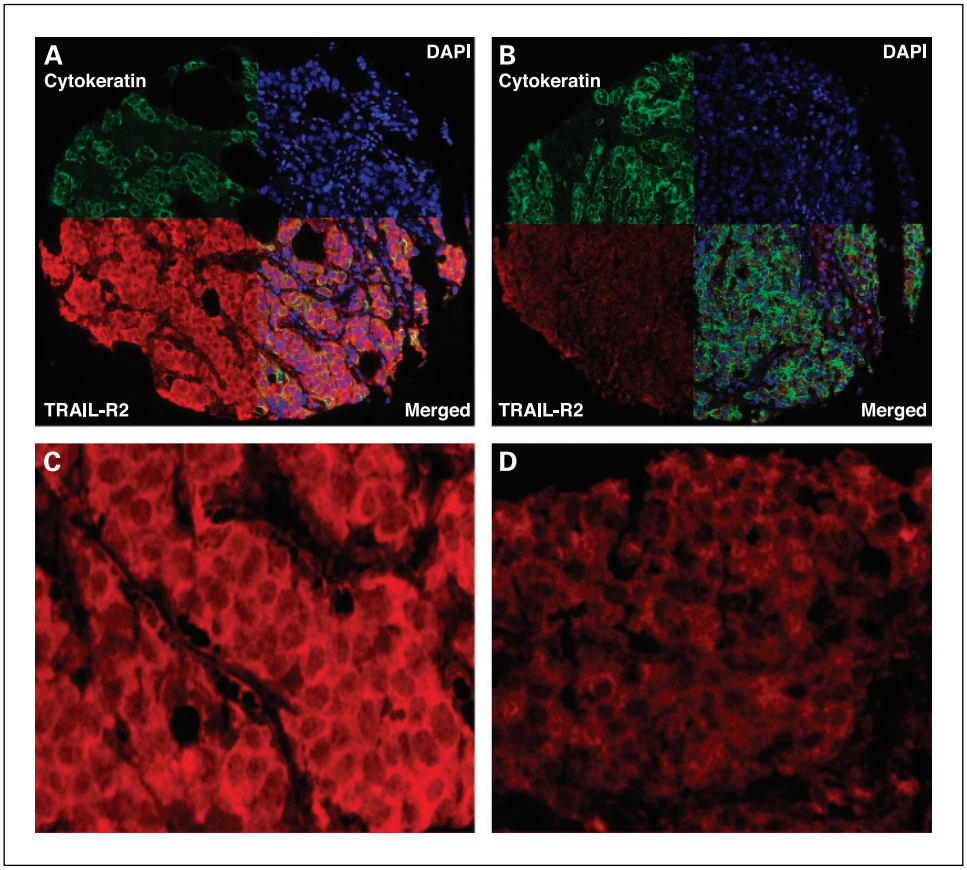

Fig. 1. High (*A*) and low (*B*) membranous TRAIL-R2 expression in a breast cancer histospot using cytokeratin to define tumor mask, 4,6-diamidine-2-phenylindole (*DAPI*) to define the nuclear compartment, and Cy5 for target (TRAIL-R2) identification at ×10 magnification. High (*C*) and low (*D*) TRAIL R-2 expression using ×60 magnification.

Table 1. Cox univariate survival analysis

Patient group	TRAIL-R1		TRAIL-R2	
	P	n	*P*	n
Node positive	0.9831	203	0.0740	251
Node negative	**0.0430**	202	**0.0205**	234
All nonmetastatic	0.1 207	405	**0.0005**	485

assess the independent predictive value of high TRAIL-R2 expression. TRAIL-R2 expression retained its independent predictive value, as did tumor size, nodal status, and age at diagnosis, as shown in Table 2.

To assess the association between TRAIL-R2 expression and other commonly used clinical and pathologic variables, we used the χ^2 test. We found a very strong association between high TRAIL-R2 expression and lymph node involvement (P = 0.0006) and a weaker association between high TRAIL-R2 expression and lack of estrogen receptor expression (P = 0.03), as shown in Table 3.

A newly developed statistical analysis software program, X-tile (21), was used to find the optimal cut point in the population of AQUA scores. The optimal cut point divided the population into two groups, placing the lower 30% of the raw, continuous AQUA scores into one group, and the upper 70% of the scores into another group. This cut point resembled the results shown in the Kaplan-Meier curves above, with scores divided into quartiles, where the upper three quartiles are grouped together and separated from the lower quartile. By dividing the population into two groups using X-tile, we continued to see a highly significant association between high TRAIL-R2 expression and decreased survival (P = 0.0003).

Because TRAIL-R2 is an attractive drug target, we assessed the differences between benign and malignant breast tissue. A subset of the breast tumor cohort, consisting of 92 patients, was simultaneously stained with 95 unmatched benign breast specimens obtained from patients undergoing breast reduction surgery. Unpaired t tests showed that TRAIL-R2 expression was significantly higher in malignant versus benign tissue cores ($P < 0.0001$).

There was no significant difference between TRAIL-R2 expressions in the lowest quartile of breast cancer patients and in the benign breast specimens (P = 0.4).

Discussion

In this study, we quantitatively assessed expression of TRAIL-R1 and TRAIL-R2 on a large cohort of primary breast cancer

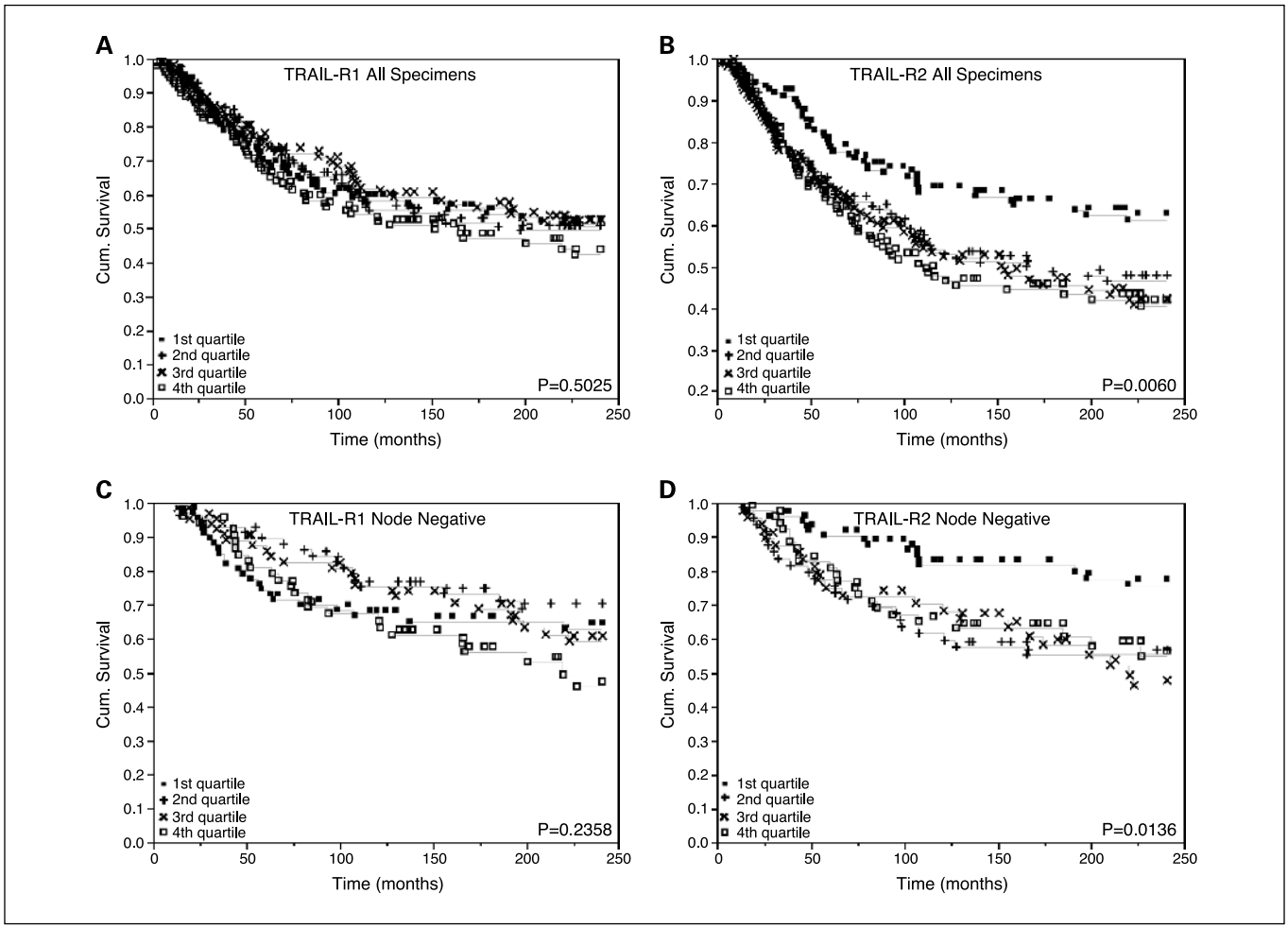

Fig. 2. Kaplan-Meier survival curves for quartiles of AQUA scores for the entire cohort of patients for TRAIL-R1 (*A*) and TRAIL-R2 (*B*), and for the node-negative subset of patients for TRAIL-R1 (*C*) and TRAIL-R2 (*D*).

Table 2. Multivariate analysis as determined by Cox regression analysis

Variable	95% Confidence interval	Relative risk	*P*
Tumor size			
>2 cm	1.477-2.82	2.041	**<0.0001**
Age at diagnosis			
<50 y	1.02-2.011	1.432	**0.0381**
Nuclear grade			
High (3/3)	0.364-1.330	1.098	0.2722
Estrogen receptor status			
Negative (0/3)	0.962-1.819	1.323	0.0855
Progesterone receptor status			
Negative (0/3)	0.885-1.607	1.192	0.2475
HER2/neu status			
Positive (2-3/3)	0.96-1.953	1.37	0.0828
TRAIL-R2 expression			
High	1.126-2.354	1.628	**0.0096**
Nodal status			
Positive	1.3-2.4	1.76	**0.0003**

specimens and evaluated the association between expression of these receptors and breast cancer specific survival. Our AQUA method gives objective, continuous measures of expression, rather than arbitrary pathologist-based divisions of staining into nominal scores of 0, 1, 2, and 3 or "positive/negative." Our results were reproducible when using a second set of arrays with different cores from tumors of the same patients.

There was no significant association between TRAIL-R1 expression and survival in the entire cohort. Among the node-negative patients only, an association between high TRAIL-R1 expression and survival was seen on Cox univariate analysis, but not when the scores were divided into quartiles and log-rank analysis was done.

High TRAIL-R2 expression was highly significantly associated with decreased survival in the entire cohort ($P = 0.0005$) and in the node-negative subset, both by Cox univariate analysis and by log-rank analysis of quartiles of scores. High TRAIL-R2 expression remained an independent predictor of survival on multivariate analysis. A strong association was found between high TRAIL-R2 expression and lymph node involvement. Moreover, TRAIL-R2 expression was significantly stronger in malignant specimens than in benign breast specimens obtained from healthy women undergoing mammoplasty.

Table 3. Association between high TRAIL-R2 expression and commonly used clinical and pathologic variables

Variable	*P* (χ^2 test)
Tumor size > 2 cm	0.59
Age at diagnosis < 50	0.988
Nuclear grade 3/3	0.125
Estrogen receptor 0/3	**0.03**
Progesterone receptor 0/3	0.0548
HER2/neu 2-3/3	0.4311
Nodal positivity	**0.0006**

Staging of primary breast cancer is important for determining prognosis and selecting patients for adjuvant chemotherapy, which reduces the risk of relapse and death. Molecular markers in the primary specimen that are highly associated with survival and/or lymph node involvement, such as TRAIL-R2, could replace or supplement standard staging information obtained from complete lymph node sampling, and improve staging for node-negative patients. In situations in which surgical staging procedures such as lymph node dissection are incomplete or inadequate, prognostic information from the initial biopsy could be useful in guiding future treatment decisions. Examples include patients for whom nodal dissection is not feasible due to financial considerations, comorbid conditions, and use of neoadjuvant chemotherapy. Moreover, given that surgical staging has limited therapeutic utility (with the exception of provision of local control for patients with massive lymphadenopathy; ref. 22), accurate molecular predictors of poor outcome based on information collectible at the time of initial biopsy might eventually obviate the need for lymph node dissections. Reliable prognostic markers could also be used to select node-negative breast cancer patients at high risk for disease recurrence, thus avoiding the toxicity associated with chemotherapy for the vast majority of patients who are cured by local therapy alone (23, 24). Similarly, such markers could be used to avoid aggressive therapy in the smaller subset of node-positive patients who are cured without additional systemic therapy (25).

Ligand binding to TRAIL-R2 induces apoptosis, hence the finding of a correlation between higher expression and decreased survival was unexpected. However, the same findings have been reported in non–small cell lung cancer, in which high TRAIL-R2 expression was also associated with decreased survival, and no association was found between TRAIL-R1 expression and survival (26). In colon cancer, strong TRAIL-R2 expression has been shown at the invasion front of the tumor (27). In our breast cancer specimens there is no equivalent to the invasion front seen in colon cancer. To date, we have not found any other studies evaluating the association between TRAIL-R2 expression in tumors and survival.

The biological basis for the association between high TRAIL-R2 expression and decreased survival has not been determined. We also showed that TRAIL-R2 expression is higher in malignant cells than in normal breast tissue. Because signaling through TRAIL-R2 leads to cell death, it is highly unlikely that a ligand-receptor interaction is contributing to tumor aggression. Sheikh et al. (28) showed that TRAIL-R2 gene expression is induced by genotoxic stress, and the induction is both p53 dependent and independent. Tumor necrosis factor-α also increases TRAIL-R2 gene expression in a p53-independent manner. Ravi et al. (29) showed that activation of nuclear factor κB (NF-κB) induces expression of TRAIL-R2. More importantly, they showed that the manner of NF-κB activation directs the cell toward a proapoptotic versus antiapoptotic state. Genotoxic stress in a p53-dependent manner, or irradiation in p53-independent manner, induced NF-κB activation and NF-κB dimers containing the c-rel subunit, which increased TRAIL-R2 expression and cell sensitivity to TRAIL-ligand induced death. In contrast, cytokines such as tumor necrosis factor-α and the growth factor heregulin also activated NF-κB, but NF-κB dimers contained the relA subunit, which induced both increased expression of TRAIL-R2 and the antiapoptotic protein bcl-X_L. Cells exposed to cytokine or growth factor stimuli became resistant to TRAIL ligand despite increased expression of TRAIL-R2. Therefore, the result of an activating TRAIL-R2 signal in cells which have increased expression of the receptor seems to depend, at least in part, on whether or not increased TRAIL-R2 expression was the result of NF-κB activation, the mechanism by which NF-κB was activated, one resulting in a proapoptotic state via the c-rel subunit and the other antiapoptotic via the relA subunit, and its effects on bcl-X_L. The preclinical studies by Shiekh et al. and Ravi et al. provide a possible explanation for the correlation between higher TRAIL-R2 expression and decreased survival in our breast cancer patient cohort. High TRAIL-R2 may be a marker of tumor cells that have increased activation of NF-κB. The association with poorer clinical outcome suggests that activation of NF-κB occurred in response to signals that favor overexpression of antiapoptotic proteins, and thus favor tumor cell survival. Examples of signals that produce antiapoptotic NF-κB activation include cytokines and growth factors or perhaps aberrant activation of pathways distal to the cytokine/growth factor receptors. In a small series of 31 patients with breast cancer, Biswas et al. measured NF-κB activation in tumor extracts and observed that NF-κB was predominantly activated in estrogen receptor–negative and erb-B2–positive tumors, which are associated with a poorer prognosis (30).

The fact that TRAIL-R2 expression was higher among the node-positive than the node-negative patients, and was associated with decreased survival within the node-negative but not within node-positive cohort, suggests that the biological events which are simultaneously permissive for metastatic behavior and also lead to high TRAIL-R2 expression occur early in the course of the disease. Although TRAIL-R2 expression was higher in patients with a poorer prognosis as well as in malignant (versus benign) breast tissue, suggesting that TRAIL-R2 might be a good therapeutic target, our data combined with the preclinical data suggest that careful strategies are needed in developing rational approaches to targeting the receptor in breast cancer. For example, high TRAIL-R2 expression resulting from antiapoptotic NF-κB activation might render cells relatively resistant to TRAIL-R2 agonists alone or, possibly, chemotherapy alone. Preclinical data have shown synergism between TRAIL or TRAIL-R2 agonists and chemotherapy in breast cancer (5, 6, 12), but the level of TRAIL-R2 expression and its relationship to cell sensitivity to the combinations have not been carefully examined. In tumors with high TRAIL-R2 expression, obtaining optimal efficacy of TRAIL-R2 agonists might require additional interventions, such as the addition of NF-κB inhibitors, inhibition of proximal cytokines or growth factors, or inhibition of downstream antiapoptotic proteins such as bcl-X_L or X-linked inhibitor of apoptosis protein. Other potential mechanisms that would allow tumor cells to survive despite high levels of TRAIL-R2 require further investigation, such as increased expression of the TRAIL decoy receptors and increased expression of Fas-associated death domain–like interleukin-1ß converting enzyme–like inhibitory proteins. Conversely, low TRAIL-R2 expressers may reflect a population of patients sensitive to TRAIL receptor agonists, and may be the best population to target with these agents alone or in combination with chemotherapy.

Thus, in addition to a potentially important role as a prognostic marker, our findings have important implications for the therapeutic application of TRAIL-R2 agonists in breast cancer (5). In addition to the metastatic disease setting, where novel therapies are clearly needed, there remains a percentage of node-negative and node-positive breast cancer patients whose disease recurs despite aggressive therapy (23, 31). These patients could benefit from the addition of novel therapies to standard chemotherapy. Patients with a good prognosis (23, 32) might also benefit, as they could have an improved risk/benefit ratio if treated with less toxic targeted therapies. The addition of TRAIL-R2 activators to current therapy or as an alternative to standard chemotherapy could improve the therapeutic ratio and outcome of patients. However, our data, as well as the emerging preclinical data, suggest that selection of patients, appropriate characterization of individual tumor biology, and perhaps intervention with additional rationally targeted agents might be necessary to achieve the best results.

In summary, our study shows a strong association between high TRAIL-R2 expression and decreased survival in primary breast cancer, both among the entire patient cohort and among the node-negative patients only. High TRAIL-R2 expression was associated with lymph node involvement. Prospective studies are needed to confirm the prognostic role of TRAIL-R2. Expression of TRAIL-R2 was stronger in tumors than in benign breast specimens. Further work is needed to elucidate the biological significance of high TRAIL-R2 expression and poor outcome, and to establish the association between TRAIL-R2 expression and response to therapy that targets this receptor. All future clinical trials including TRAIL-R2 agonists for breast cancer should stratify patients based on TRAIL-R2 expression.

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