Research Article

Cancer Epidemiology, Biomarkers & Prevention

TLE3 Expression Is Associated with Sensitivity to Taxane Treatment in Ovarian Carcinoma

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Abstract

Background: We have previously shown that transducin-like enhancer of split 3 (TLE3) is associated with outcome specifically in patients with taxane-treated breast cancer and not in patients treated with anthracycline-based regimens without a taxane. The purpose of this study was to assess the association between TLE3 expression and recurrence in patients with ovarian carcinoma treated with a taxane containing regimen as opposed to those treated with a platinum-based agent alone.

Methods: We carried out immunohistochemical staining of TLE3 in two series of ovarian cancer specimens from the University of Alabama at Birmingham, Birmingham, AL and the Royal Hospital for Women, Sydney, Australia. Local and distant recurrences within the first five years of follow-up were analyzed using Kaplan–Meier, Cox proportional hazard, and multivariate analysis to assess an association between TLE3 expression and response to therapy.

Results: TLE3 was expressed in approximately 30% of tumors and expression was associated with a favorable outcome only in patients who had received taxane as part of their treatment regimen ($n = 173$, HR $= 0.62$, $P = 0.012$; $P_{\text{interaction}} = 0.024$). Further analysis revealed that the predictive association between TLE3 expression and outcome was strongest in patients with nonserous histology.

Conclusion: High TLE3 expression predicts a favorable response to taxane containing chemotherapy regimens in ovarian carcinoma.

Impact: Our findings warrant an independent evaluation of TLE3 as a potential therapeutic response marker for taxane-based chemotherapy in ovarian cancer. *Cancer Epidemiol Biomarkers Prev; 21(2); 273–9.* ©*2011 AACR.*

Introduction

Transducin-like enhancer of split 3 (TLE3) is a transcriptional repressor that interacts with a chromatin complex acting downstream of adenomatous polyposis coli (APC) and β-catenin in the Wnt pathway (1). The TLE family has been implicated in tumorigenesis and has been shown to interact with and modulate the Notch pathway, via phosphorylation by mitogen-activated protein kinase (MAPK) in response to epidermal growth factor receptor (EGFR) signaling (2, 3).

TLE3 was first identified as a candidate biomarker of taxane sensitivity in breast cancer in a large screen of candidate immunohistochemical (IHC) classifiers in a community cohort study (4). TLE3 was one of several biomarkers found to be prognostic in all patients; however, the association with outcome was present only in patients who received treatment with either a taxane or a methotrexate containing regimen. The hypothesis that TLE3 expression was associated with response to taxane therapy was subsequently tested by conducting a retrospective validation study in a triple negative (estrogen receptor, progesterone receptor, and ERBB2 negative), uniformly high-grade breast cancer cohort. TLE3 expression was confirmed to be associated with favorable outcome in taxane-treated patients and, as predicted, there was no association with outcome in patients treated with anthracyclines only, regardless of clinical stage at diagnosis (5).

Epithelial ovarian carcinoma is an aggressive malignancy, with >65% of patients diagnosed at advanced stage, when the cancer has spread into the peritoneal cavity. The 5-year survival rate after initial diagnosis for

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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doi: 10.1158/1055-9965.EPI-11-0917

late-stage patients is <30%, compared with a 5-year survival rate of >90% for patients diagnosed at early stage (6, 7). Current treatment for ovarian cancer includes surgical debulking followed by a standard chemotherapy regimen. Recommended regimens usually include a platinum-based agent in combination with a taxane class agent. Paclitaxel was originally reported as a treatment for platinum-resistant disease; trials showed that taxane/platinum combination therapy resulted in improved response rates as well as progression-free and overall survival in patients with ovarian cancer (reviewed in ref. 8). Despite this improved response, 20% to 30% of patients with ovarian cancer harbor residual or progressive disease during treatment (6). Furthermore, treatment with taxanes is associated with a significantly increased incidence of serious side effects including myelosuppression, peripheral neuropathy, and hypersensitivity reactions (9). The identification of biomarkers that could identify which patients would most significantly benefit from taxane containing regimens, and conversely those likely not to benefit but still suffer the morbidity associated with treatment would significantly help in patient management. In this study, we sought to examine the association of TLE3 expression with outcome in 2 cohorts of women with epithelial ovarian cancer (Royal Hospital for Women, Sydney, Australia and University of Alabama at Birmingham, Birmingham, AL).

Materials and Methods

Patient samples and assembly of clinical data sets

Institutional ovarian cancer cohorts from the University of Alabama at Birmingham (UAB) and the Royal Hospital for Women (RHW) were used in this study. In all cohorts, patient tumor paraffin blocks were assigned an anonymous unique identifier linked to clinical databases that contained treatment and outcome data. Institutional Review Board approval was obtained for the use of patient blocks at each respective institute. A total of 583 cases were originally made available for this study (346 UAB patients and 237 RHW patients). Patients were excluded if they had received neoadjuvant chemotherapy or were enrolled in a clinical trial (as they may have received treatment with a novel drug whose effects could confound the results). In addition, we excluded patients who had progressive disease, patients without follow-up data and patients whose TLE3 staining was unscoreable due to technical issues (Supplementary Fig. S1). Thus, a total of 296 patients were analyzed in this study (135 UAB patients and 161 RHW patients). Response to therapy in both cohorts was assessed by the absence of local or distant recurrence, as defined by raising CA-125 levels or as detected by radiological methods.

Tissue arrays, immunohistochemistry, and scoring

The UAB cohort tissue microarray (TMA) blocks each contained single 0.6-mm cores sampled from representative paraffin blocks from each patient. The RHW TMA was constructed at the Garvan Institute for Medical Research (Sydney, Australia) using triplicate cores from each patient in a single block. Staining methods and scoring criteria are as described earlier (5). Briefly, slides were deparaffinized by submersing in xylene 3× 10 minutes and rehydrated by rinsing 3× in 100% ethanol and 2× in 95% ethanol. Antigen retrieval was conducted by boiling in a microwave for 11 minutes in 10 μmol/L buffered citrate (pH 6.0). Slides were blocked in 0.03% hydrogen peroxide and stained using antibody diluted to appropriate titer in Dako Diluent (DakoCytomation) for 1 hour at room temperature. As a control for staining quality and to select titer, candidate dilutions were first tested on a small "titer" tissue array that contained positive and negative breast cancer cases and tumor-derived cell lines suspended in paraffin. IHC analysis for TLE3 was conducted using a polyclonal affinity-purified antibody at a titer of 1:1,200 (RHW) to 1:700 (UAB). Secondary antibody was applied for 1 hour, and staining was visualized by the DakoCytomation Envision Staining Kit in accordance with the instructions of the manufacturer. TLE3 stains nuclei with variable intensity, however, in stained cases most tumor cell nuclei stain uniformly. Cores were manually scored by a trained cytotechnologist, under supervision of a pathologist, and considered positive if greater than 30% of nuclei stained regardless of staining intensity. Replicate scores for a single case were compressed by assuming the maximum score (for 1–3 replicate cores, or by using the rounded average score for $\geq$4 replicate cores).

Statistical considerations

The relationship between TLE3 expression and recurrence within 5 years of diagnosis was analyzed with S-plus software (Tibco Software Inc.). Kaplan–Meier and log-rank analysis were used to analyze the association of TLE3 expression and recurrence-free survival. Interactions between TLE3 expression and taxane treatment are reported as a multivariate analysis wherein TLE3 expression, inclusion of taxane, and the interaction term were assessed simultaneously by Cox proportional hazards. All reported P values are two sided.

Results

Patient characteristics and TMA staining for TLE3 expression

Table 1 describes the clinical characteristics for the RHW and UAB patient cohorts analyzed in this study. In the UAB cohort, 129 of 135 (96.7%) patients were treated with a regimen containing a taxane and a platinum class agent whereas in the RHW cohort 64 of 161 (39.8%) patients were treated with a regimen containing a taxane while the remainder were untreated, treated with a platinum class agent alone or unspecified (Table 1).

Tissue arrays were constructed from surgical resection specimens in paraffin blocks from patients with epithelial ovarian carcinoma. Staining was carried out with anti-TLE3 monoclonal antibody and scored using criteria

Table 1. Clinical characteristics for the UAB and RHW patient cohorts

	UAB	RHW
Treatment		
Taxol	129	64
Other chemotherapy	4	40
None	2	57
Diagnosis		
Serous	53	118
Other nonserous	19	20
Endometrioid	41	13
Clear cell	2	10
Not specified	20	0
Stage		
1	11	57
2	24	10
3	90	80
4	10	11
Not specified	0	3
Grade		
Borderline	0	42
1	5	11
2	27	34
3	61	62
Not specified	42	12
Debulking status		
Optimal	84	133
Suboptimal	39	17
Not specified	12	11
Mean recurrence, d	594	704
TLE positive	32%	30%

established in prior breast cancer studies, with cores considered positive if ≥30% of nuclei stained positive for TLE3 (5). Figure 1 shows representative examples of TLE3 staining in serous, clear cell, and endometrioid cases from the UAB cohort. Table 1 shows the number of patients in each cohort that stained positive for TLE3 expression. Across both cohorts, 92of 296 (31%) patients expressed TLE3 (32% UAB and 30% RHW). There was no significant difference in TLE3 expression between serous and nonserous histotypes (χ^2 P value = 0.36).

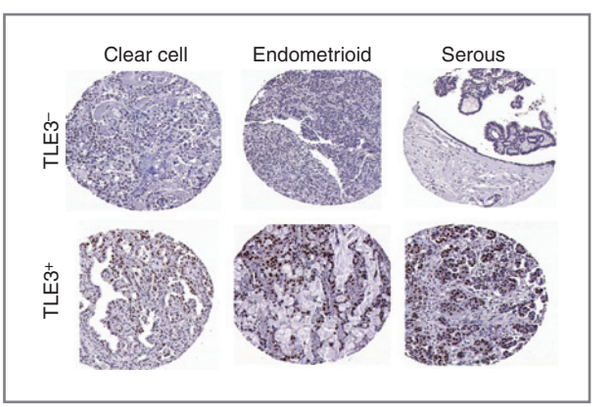

Figure 1. IHC staining of ovarian carcinoma specimens with anti-TLE3 monoclonal antibody. Examples of positive and negative staining for TLE3 in serous, clear cell, and endometrioid histotypes. A total of 600-μm cores are shown at 40× magnification. Staining was considered positive if >30% nuclei stained, regardless of intensity.

Association between TLE3 expression and outcome

Table 2 and Fig. 2 describe the association between TLE3 expression and outcome (5-year recurrence) in the patient cohorts, stratified by taxane treatment, institution, and tumor histology. Across both cohorts, TLE3 expression was significantly associated with recurrence in taxane-treated patients (n = 173, HR = 0.62, P = 0.012), whereas there was no relationship with decreased recurrence in patients treated with a platinum agent only or untreated (n = 100, HR = 1.53, P = 0.22; Fig. 2A and B). An interaction test to assess the apparent differential response to therapy based on TLE3 expression (TLE3: taxane) was significant (P = 0.024). The interaction remained significant when adjusted for grade, stage, and debulking status, whether all factors are assessed individually or together. TLE3 expression was significantly associated with outcome in taxane-treated patients in the UAB cohort (n = 110, HR = 0.64, P < 0.05) but not the RHW cohort (Fig. 2C and D, Table 2). There was no relationship between TLE3 expression and outcome in either untreated patients or in patients treated with regimens that did not contain a taxane (Fig. 2E and F, Table 2).

Because recent studies have shown that different histologic subtypes of ovarian cancer represent molecularly distinct diseases (reviewed in ref. 10), we wished to examine the association between TLE3 expression and recurrence across histotypes of ovarian tumors. Examination of the relationship of histologic subtype with TLE3 expression and outcome revealed that the association between TLE3 expression, taxane treatment, and outcome was most strongly present in samples with nonserous histology (n = 69, HR = 0.25, P < 0.0001; Fig. 2G and H). This included mostly tumors with mucinous and endometrioid histology as well as more rare variants such as clear cell carcinoma. The strong association between TLE3 expression and outcome in patients with nonserous histology was significant in the UAB cohort (n = 58, HR = 0.27, P = 0.0007) but not the RHW cohort (Fig. 2I and J, Table 2). This difference is likely due to the limited number of nonserous cases in the RHW cohort. As serous histotypes make up the majority of ovarian cancer, the combined cohorts were underpowered to determine whether the association with response to taxane treatment was more prevalent in any subtype of the nonserous tumors.

Discussion

Advanced epithelial ovarian cancer is an aggressive tumor with only moderate response to cytotoxic therapy and few effective options in refractory or recurrent

Table 2. Association between TLE3 expression and 5-year recurrence in the patient cohorts

	HR	*P* value	TLE3$^+$	TLE3$^-$	*N*
Both cohorts					
Nonserous					
Taxane$^+$	**0.25**	<**0.0001**	28	41	69
Taxane$^-$	1.95	0.33	8	25	33
Serous					
Taxane$^+$	1.12	0.6	31	73	104
Taxane$^-$	1.4	0.4	19	48	67
All patients					
Taxane$^+$	**0.62**	**0.01**	59	114	173
Taxane$^-$	1.53	0.22	27	73	100
RHW					
Nonserous					
Taxane$^+$	0.18	0.14	6	5	11
Taxane$^-$	2.1	0.34	6	23	29
Serous					
Taxane$^+$	0.87	0.68	16	36	52
Taxane$^-$	1.36	0.45	19	47	66
All patients					
Taxane$^+$	0.577	0.095	23	41	63
Taxane$^-$	1.5	0.26	25	70	95
UAB					
Nonserous					
Taxane$^+$	**0.27**	**0.0007**	22	36	58
Taxane$^-$	>10	0.96	2	2	4
Serous					
Taxane$^+$	1.45	0.29	15	37	52
Taxane$^-$	1	1	0	1	1
All patients					
Taxane$^+$	**0.64**	**0.046**	37	73	110
Taxane$^-$	>10	0.24	2	3	5
Multivariate models					
Both cohorts					
TLE3	1.45	0.22			273
Taxane	3.3	<**0.0001**			
TLE3:taxane	0.42	**0.024**			
RHW					
TLE3	1.48	0.26			158
Taxane	3.18	<**0.0001**			
TLE3:taxane	0.39	0.054			
UAB					
TLE3	2.4	0.54			115
Taxane	2.9	0.3			
TLE3:taxane	0.25	0.34			

NOTE: Bold values indicate statistically significant associations.

disease (11). The recommended therapy for first line treatment has not changed significantly in the past decade and includes a taxane agent combined with a platinum class agent. Yet, with recurrence rates of greater than 70% in late stage disease and the frequent development of chemotherapy resistance, the development of new therapeutic approaches remains a priority.

TLE3 was originally identified as a candidate taxane response biomarker in a community cohort study of breast cancer (5). This observation was validated in a retrospective study of triple negative breast cancer at the Roswell Park Cancer Institute (Buffalo, NY), which found that TLE3 expression was associated with disease-free survival in patients who had received

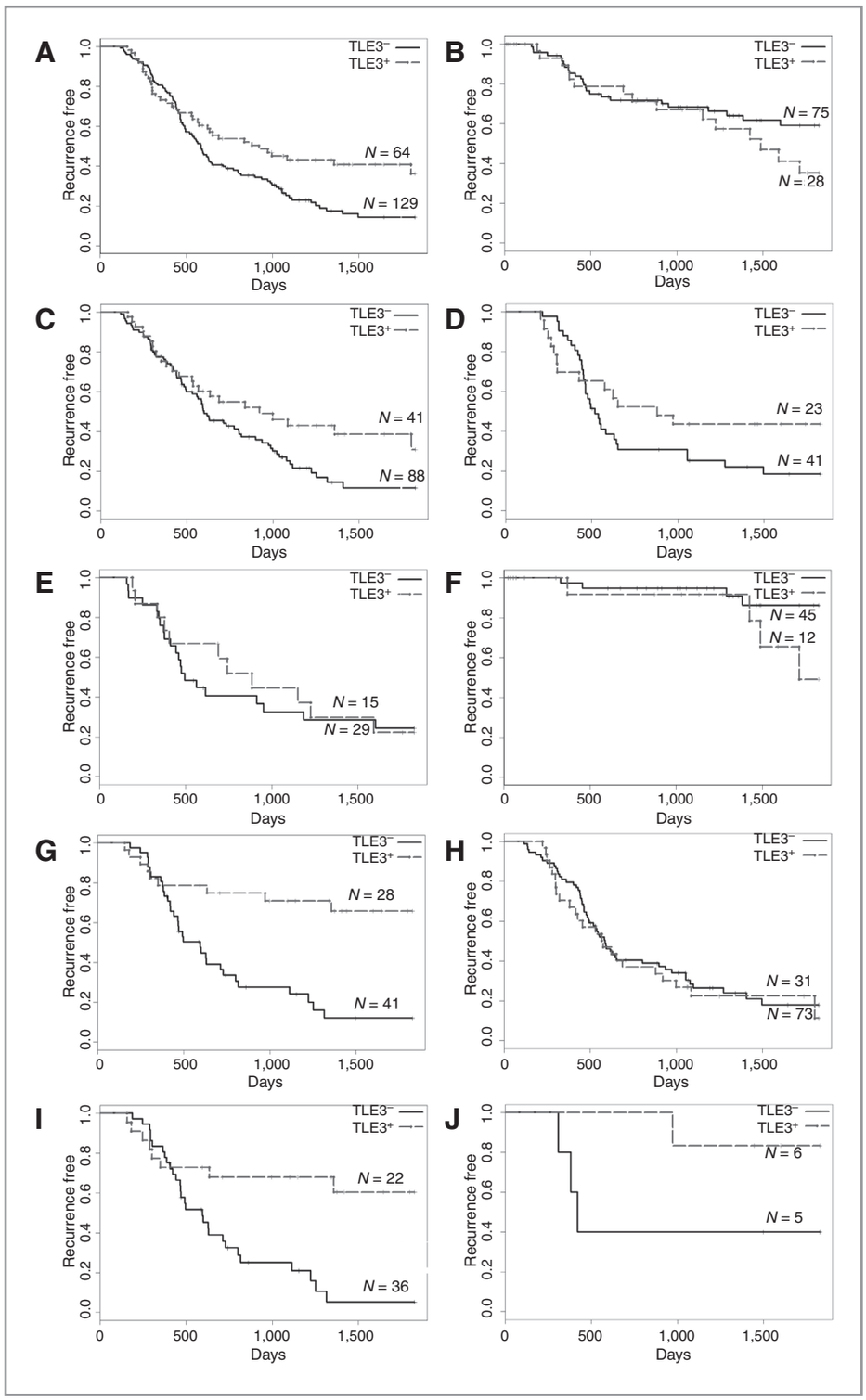

Figure 2. Kaplan–Meier plots depicting outcomes in TLE3 expressing (- - - -) and not expressing (—) patient subsets. A, all patients in UAB and RHW cohorts treated with a taxane containing regimen; B, all patients in UAB and RHW cohorts treated with platinum agent only or untreated. C, UAB cohort treated with a taxane containing regimen; D, RHW cohort treated with a taxane containing regimen. E, all patients treated with regimens that did not contain a taxane. F, all patients that did not receive adjuvant chemotherapy. G, Nonserous histology patients treated with a taxane containing regimen. H, serous histology patients treated with a taxane containing regimen. I, UAB nonserous histology patients treated with a taxane containing regimen. J, RHW nonserous histology patients treated with a taxane containing regimen.

taxane-based treatment (5). In a separate study, we have also found TLE3 expression to be predictive of response to taxane therapy in non–small cell lung cancer (NSCLC; Alex Soltermann, personal communication).

In this current study in patients with ovarian cancer, we show that TLE3 expression is predictive of 5-year recurrence in advanced epithelial ovarian cancer. Further analysis suggests that the relationship between TLE3

expression and outcome was strongest in nonserous cases. As genetic and molecular studies have revealed that different ovarian cancer histotypes present distinctive gene expression and mutation patterns, it is not surprising that nonserous tumors expressing TLE3 respond uniquely to taxane therapy. Indeed, endometrioid ovarian cancers generally respond better to platinum and taxane-based treatments than do clear cell and mucinous ovarian cancers (12).

TLE3 is a mammalian homologue of the drosophila groucho genes which have been shown through genetic analysis to interact with the Notch and Wnt pathways implicated in the control of epithelial differentiation (1). The Wnt pathway has been most extensively studied in the context of the hereditary mutation of APC or β-catenin that causes the familial cancer syndrome hereditary nonpolyposis colorectal cancer (HNPCC). Somatic mutations in the Wnt pathway are also frequently found in epithelial ovarian cancer, most commonly in those with endometrioid differentiation (13). Groucho family members are transcriptional repressors which act in the Wnt pathway downstream of the APC and β-catenin by binding to histone deacetylases as well as other members of transcriptional complexes including T-cell factor/lymphoid enhancer factor (TCF1/LEF). Targets of these complexes include a number of oncogenes, notably cyclin D1 (14). Targeted disruption of the APC or β-catenin genes in cell line and mouse models result in cytoskeleton abnormalities, aberrant growth patterns, and other tumorigenic phenotypes (13, 15). It is interesting that taxane sensitivity in this study was found mostly in nonserous tumors reminiscent of the predominance of somatic mutations of APC or β-catenin in epithelial ovarian cancer primarily in cases with endometrioid histology (16). However, in our study, the association between TLE3 expression and response to taxane therapy was not limited to those with endometrioid differentiation, and therefore, this finding requires further study in a larger cohort.

Taken together, the consistent finding of a predictive relationship between TLE3 expression and response to taxane therapy in breast, lung, and ovarian carcinoma is compelling evidence for TLE3 as a biomarker of sensitivity to treatment with taxane therapy in carcinoma. Studies have shown that the Wnt pathway regulates cytoskeletal activity, and aberrant Wnt signaling impacts chromosome segregation and spindle orientation (17, 18). It is thus plausible that TLE3 is acting as an indicator of Wnt pathway activity which impacts cytoskeletal integrity and in turn taxane sensitivity. Clinical cohorts populated with nonserous ovarian carcinomas are rare and the small numbers in the cohorts we have examined in this study provide weak power for the analysis of association between TLE3 expression and outcome in nonserous histotypes. Therefore, we suggest that prospective trials of taxane regimens stratified by TLE3 expression in ovarian carcinoma would be an important follow-up to this study. Clinical trials that have randomized patients to receive a taxane agent have been carried out in breast and NSCLC, and the data are available for correlative science studies (19–21). The study of TLE3 in these different carcinoma types should be carried out to further examine TLE3 as a clinically useful biomarker of response to taxane treatment.

Disclosure of Potential Conflicts of Interest

B.Z. Ring, D.T. Ross, and R.S. Seitz have employment in Clarient, Inc. D.T. Ross and R.S. Seitz received a commercial research support. No potential conflicts of interest were disclosed by the other authors.

Grant Support

This research was supported in part by the Gynaecological Oncology (GO) Fund of the Royal Hospital for Women Foundation, Sydney, Australia; Cancer Australia; Cancer Institute New South Wales (NSW); National Health & Medical Research Council of Australia; and the RT Hall Trust.

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Received September 28, 2011; revised November 29, 2011; accepted December 13, 2011; published OnlineFirst December 22, 2011.

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