SPANDIDOS PUBLICATIONS

Categorical meta-analysis of Osteopontin as a clinical cancer marker

GEORG F. WEBER[1,2], G. SCOTT LETT[3] and NED C. HAUBEIN[3]

[1]James L. Winkle College of Pharmacy, University of Cincinnati Academic Health Center; [2]MetaMol Theranostics, Cincinnati, OH; [3]The BioAnalytics Group, Jamesburg, NJ, USA

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Abstract. Although extensive literature exists on cancer biomarkers few have found entry into clinical use. In particular, the cancer metastasis gene Osteopontin has been investigated extensively but it has not yet been applied to routine diagnostics. Here, we conduct a meta-analysis of data from the published literature and from RNA microarrays deposited in Oncomine. Osteopontin has been associated with 34 cancers. It is a marker for breast, cervical, colorectal, head and neck, liver, lung, ovarian and prostate cancers, as well as for sarcoma. Osteopontin is overexpressed in the metastases of colorectal cancers, lung cancers and melanomas, but not in ovarian cancer. Further, Osteopontin is indicative of the underlying mechanism of transformation only in certain virally induced tumors, where its function as a TH_1 cytokine likely plays important roles. These results refine the value of Osteopontin as a cancer biomarker.

Introduction

In clinical oncology, there is a lack of biomarkers that distinguish highly aggressive tumors from moderately aggressive and non-aggressive ones. Specifically, few markers that predict invasiveness have been firmly established. Better molecular predictors of cancer progression are needed to facilitate rational treatment decisions. One candidate marker for the progression of various malignant tumors has been Osteopontin. The association of this molecule with cancer was initially reported in 1979 (1) and has been under study since the 1980s. Osteopontin is a secreted glycophosphoprotein that physiologically may serve as a cytokine and as an extracellular matrix molecule. In cancer, it can support cell migration and protect from programmed cell death after the ligation of certain integrin receptors or CD44 splice variants on the tumor cell surface. These functions can enhance metastasis formation.

Although numerous studies have investigated Osteopontin as a cancer marker in patient specimens, the published information has not been analyzed comprehensively. We have used categorical meta-analysis to show that Osteopontin is a marker for overall and disease-free survival as well as tumor stage, tumor grade and early tumor progression in subsets of 34 different cancers (2). In addition, multiple other aspects of cancer have been associated with this molecule. Here we study the correlation of Osteopontin with the presence of cancer, primary vs. metastatic cancers, and cancer risk and etiology.

Materials and methods

Data extraction. A Medline search with the keywords 'Osteopontin and cancer' through December 2008 resulted in 800 hits. Titles and abstracts were screened for studies involving human subjects, yielding 271 papers for initial analysis. Thirty-six articles (including reviews, commentaries, experiments only on cell lines, no results on cancer, etc.) did not contain new data on Osteopontin in human cancer. Four articles were not obtained, even after request through interlibrary loan. Three articles were excluded because they contained one retraction, one paper that pooled diverse primary tumors without separating them by tumor type and one paper that applied scientifically questionable methodology (bidigital O-ring test). This left 228 publications to be used for data extraction (2). Of foreign language articles, only the abstracts (not the full texts) were drawn on for obtaining data. For data extraction, numbers from the article text were applied directly, data presented in the format of graphs were digitized and converted to the relevant units.

The cancers covered by the original publications include: breast cancer (34), ovarian cancer (25), liver cancer (21), lung cancer (20), head and neck cancer (15), colorectal cancer (14), gastric cancer (14), prostate cancer (13), bone cancer (9), oral cancer (9), melanoma (9), pancreatic cancer (8), renal cancer (8), esophageal cancer (7), glioma (7), mesothelioma (7), thyroid cancer (7), endometrial cancer (6), myeloma (6), cervical cancer (4), gestational trophoblastic tumor (4), leukemia/lymphoma (3), granular cell tumor (2), non-melanoma skin cancer (2), ampullary cancer (2), bladder cancer (2), medulloblastoma (2), soft tissue tumors (2), teratoid

Correspondence to: Dr Georg F. Weber, James L. Winkle College of Pharmacy, University of Cincinnati Academic Health Center, 3225 Eden Avenue, Cincinnati, OH 45267-0004, USA
E-mail: georg.weber@uc.edu

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tumor (2), adrenocortical cancer (1), neuroblastoma (1), pilomatricoma (1), renal pelvis cancer (1), von Hippel-Lindau disease (1). The numbers in parentheses indicate the number of publications for each type of cancer. Note that several papers contain data on more than one type of cancer and are counted here for each. Therefore the sum is larger than the 228 original publications used for the data extraction.

Data analysis. The correlation between Osteopontin expression levels and the clinical variables of interest was examined with a traditional meta-analysis approach (utilizing absolute variable values) and also with a novel categorical approach (using ranked values). A significance level of 95% ($p<0.05$) was applied to all studies.

Effect size. One traditional technique of meta-analysis is the determination of effect sizes between two variables. We used Cohen's d (3,4) to measure effect size, calculated according to Equation 1, where the subscripts refer to two distinct sets of patients differing by grade or stage, $\bar{x}$ is the mean value for the set, n is the number of patients in the set, and s is the standard deviation. When calculating the mean and standard deviation of the Osteopontin values for each set, the sample size for each study contributing to that set was used as a weight.

$$d = \frac{\bar{x}_1 - \bar{x}_2}{S_{pooled}}, \quad S_{pooled} = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2}} \qquad [1]$$

Data ranking. Within a study, tumor stage and grade were ranked from low to high and then normalized by the number of examples in the study. Reports that combined a range of grades were assigned the mean grade. Also within a study, the Osteopontin scores were ranked from low to high. In the case of immunohistochemistry scores that reported graded results on a 0-3$^+$ scale, a composite score for the study was computed by weighting each score by the fraction of patients reported for that score. For studies using an expanded scoring system, the scores were grouped at low, medium and high levels and treated in the same way as the 0-3$^+$ results. For studies that only reported mean or median results, the raw values were simply ranked. Ranking accomplishes a self-normalization within each study (5,6) and permits the simultaneous analysis of both the summary results (mean, median only) and various graded results. In the case of immunohistochemistry, this reduces the effects of different pathologists scoring the samples. In other assay types, such as ELISA or quantitative RT-PCR, this eliminates the need for a normal standard under the assumption that all samples within a study are compared against the same standard.

Test for independence of ranked data. We utilized the Pearson χ^2 test (7,8) for independence to assess whether the Osteopontin ranks are independent of the tumor grade or stage ranks. This test was carried out by constructing contingency tables using the ranks for each variable, and populating each cell with the total number of patients reporting that combination of ranks. Separate tables were constructed for sets of studies with 2, 3 or more ranks to avoid structural zeros. Equation 2 computes a χ^2 statistic for each of the tables, where O is the number of patients in a given cell and N is the total number of patients. The degrees of freedom for each contingency table were computed as $(r-1)^*(c-1)$ where r is the number of rows and c is the number of columns. A combined χ^2 statistic for all of the individual tables was calculated by summing the sub-table χ^2 statistics and degrees of freedom.

$$\chi^2 = \sum_{i=1}^{r}\sum_{j=1}^{c}\frac{(O_{i,j} - E_{i,j})^2}{E_{i,j}}, \quad E_{i,j} = \frac{\sum_{k=1}^{c} O_{i,k} * \sum_{k=1}^{r} O_{k,j}}{N} \qquad [2]$$

Test for linear and non-linear trends of ranked data. The Mantel-Haenszel χ^2 test (7,8) was used to assess the hypothesis that the ranking of a particular clinical variable within a study is linearly related to the Osteopontin level. We utilized the same contingency tables constructed for the Pearson test for independence and applied Equation 3, where n is the total number of patients contained in the table, r^2 is the Pearson correlation between the row variable and the column variable, and M^2 is a test statistic that has an asymptotic χ^2 distribution with 1 degree of freedom. In this linear model, all patients at a given Osteopontin rank are assigned to the nearest clinical variable rank predicted by a linear fit to the observed data.

$$M^2 = (n-1) * r^2 \qquad [3]$$

We then tested for a non-linear trend by examining the residuals between the observed values and a linear model of the data. Equation 4, computed for each sub-table, assesses the significance of non-linear effects. The variables in this equation are the observed values O, the predictions of the linear model L, and the expected values defined in Equation 2. In the case of a good linear fit, the numerator approaches zero and one can conclude that only the linear effects are significant.

$$\chi^2 = \sum_{i=1}^{r}\sum_{j=1}^{c}\frac{(O_{i,j} - L_{i,j})^2}{E_{i,j}} \qquad [4]$$

Where a linear association is found between the Osteopontin ranks and the ranks of relevant clinical variables, this event is only considered to be meaningful if no non-linear trend is identified. If non-linear effects also contribute significantly, one can conclude that the Osteopontin levels and clinical variables are dependent on one another, but the type of interaction cannot be specified with confidence.

Rank based test for prediction. Receiver operator characteristic (ROC) curves are commonly used to assess diagnostic performance, which is the predictive power of one value for another. The most common feature used to quantify this characteristic is the area under the curve (AUC), which can be interpreted here as the probability that for two randomly chosen samples, the one with the higher Osteopontin rank

 have a higher rank for the clinical variable in (9). In the case of the ranked data in this study, that probability can be calculated directly. Each pair of patient groups in the study was examined, and the fraction of those where a group with higher clinical variable rank also had a higher Osteopontin level rank is reported here. The statistical significance of this fraction was tested by carrying out a Monte Carlo simulation to estimate the distribution of fractions expected for random ranks.

Reporting standards. We assessed whether the data applied to this study were skewed by publication bias according to a funnel plot analysis. The present study has been conducted according to the standards of the PRISMA Statement (10).

Conventional meta-analysis. We extracted microarray data for the *spp1* gene (Osteopontin) from Oncomine (11) with no threshold for gene rank, a threshold of 0.001 for p-value, and limited to mRNA arrays (cut-off 10/2009). The meta-analysis function contained in the software (Oncomine 4.2, www.oncomine.com) was applied. Various data sets were compared according to the rank for a gene, which is the median rank for that gene across each of the analyses. The p-value for a gene is its p-value for the median-ranked analysis.

Meta-analysis from the literature may be compromised by publication bias in favor of significant differences between study group and control group (the 'file drawer problem'). As the microarray data were deposited without specific focus on Osteopontin, the evaluation of the Oncomine data can control for potential bias in the evaluation of the literature data.

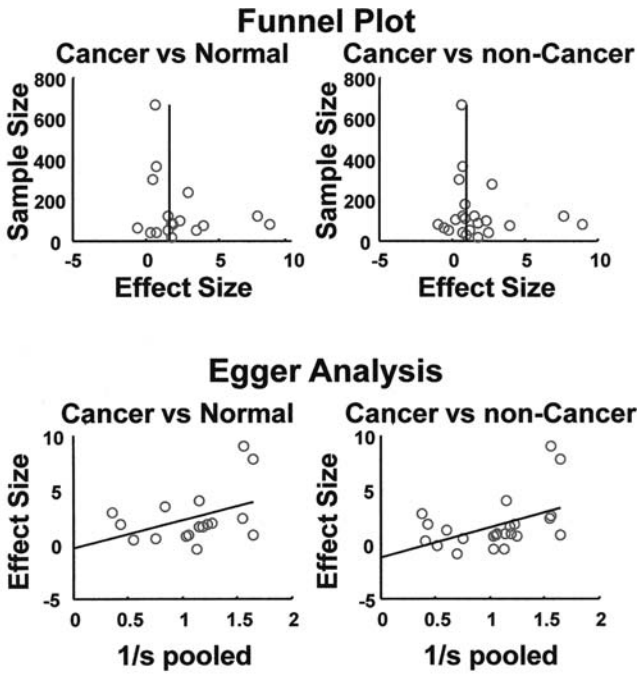

Figure 1. Test for publication bias. Funnel plots consist of some measure for the precision of the study estimate on the vertical axis, while on the horizontal axis some measure of the treatment effect is plotted. We chose to use effect size for the horizontal axis and sample size on the vertical axis. Funnel plots for the comparison of cancer against normal and non-cancer, respectively are relatively symmetrical, with the exception of two small studies with large effect sizes, indicating no evidence of publication bias. The regression line highlights the overall effect size. We also plotted the standardized estimate of effect size vs. the inverse of the pooled standard error (1/s pooled). We then performed a linear regression (the line represents the best fit linear model) and tested if the y-intercept was significantly different from 0, which would suggest the presence of bias. In this case, intercepts of -0.4 and -1.2 were both found to be statistically indistinguishable from 0 at the 90% confidence level, again finding no evidence of publication bias.

Results

We have reported that the abundance of Osteopontin correlates with poor prognosis as well as with stage, grade and progression in multiple cancers (2). The comprehensive literature and microarray (Oncomine) data available have also associated Osteopontin with other aspects of cancer. Here we evaluate those associations.

Osteopontin as a cancer biomarker. The value of Osteopontin as a diagnostic is basically dependent on the ability to distinguish cancer patients from cancer-free individuals. We therefore assessed the specificity of Osteopontin as a cancer marker based on reports in the literature. The data applied to this study were not skewed by publication bias according to a funnel plot analysis (12) (Fig. 1).

According to a conventional meta-analysis approach using Cohen's d, the mean Osteopontin levels for cancer samples were compared to both normal samples and non-cancer samples, which include healthy controls, pre-cancers and benign tumors. When the effect size was calculated for the entire data set (all cancer samples vs. all normal samples or all cancer samples vs. all non-cancer samples), effect sizes of 1.69 and 1.02 were found, respectively (data not shown). This indicates a large effect and is in agreement with our finding in ranked data of a significant trend for Osteopontin to serve as a cancer marker on the protein and RNA levels (Table IA). We utilized the Pearson χ^2 test for independence to assess whether the Osteopontin ranks were independent between cancers and non-cancers. This test was carried out by constructing contingency tables using the ranks for each variable, and populating each cell with the total number of patients reporting that combination of ranks. The Mantel-Haenszel χ^2 test was used to assess the hypothesis that the ranking of a particular clinical variable within a study is linearly related to the Osteopontin level. Of note, while Osteopontin appears to be a cancer biomarker for 31 individual malignancies (Table IB) its levels are significantly reduced below normal in non-melanoma skin cancer and gestational trophoblastic tumor. This suggests a unique role for Osteopontin in these two malignancies.

The meta-analysis function in Oncomine identified elevated Osteopontin as associated with 10 of 16 cancers (Table IC). For renal cancer, the p-value was below 0.05 for up-regulation as well as for down-regulation of Osteopontin. If both aspects were to be analyzed together there would likely be no statistically significant difference. Mostly, the literature evaluation and Oncomine are in agreement: high levels of the marker Osteopontin occur in liver, breast, colorectal, esophageal, lung, cervical, ovarian, head and neck, and prostate cancer, as well as in sarcomas. However,

Table I. Osteopontin as a cancer biomarker.

A,	Studies	Patients	Pearson χ^2	Pearson p-value	Linear χ^2	Linear p-value	Correlation coefficient	Non-linear χ^2	Non-linear p-value
Protein measures	94	10407	11028.64	<0.001	7293.04	<0.001	0.84	5677.46	<0.001
RNA measures	71	3119	3641.49	<0.001	2681.37	<0.001	0.93	1711.80	<0.001

B, Cancer type	Studies	Patients	Pearson p-value	Linear p-value	Correlation coefficient	Non-linear p-value
All	165	13526	<0.001	<0.001	0.84	<0.001
Ampullary	3	140	<0.001	<0.001	0.86	<0.001
Bladder	1	78	<0.001	<0.001	1	1
Bone	2	85	<0.001	<0.001	1	N/A
Breast	13	743	<0.001	<0.001	0.87	<0.001
Cervical	4	654	<0.001	<0.001	1	1
Colorectal	10	675	<0.001	<0.001	0.95	<0.001
Endometrial	4	214	<0.001	<0.001	0.74	1
Esophageal	5	215	<0.001	<0.001	0.94	<0.001
Gastric	11	740	<0.001	<0.001	1	1
Gest. troph.	5	88	<0.001	<0.001	-0.95	N/A
Glioma	1	33	<0.001	<0.001	1	1
Gran. cell	2	50	<0.001	<0.001	1	N/A
Head and neck	5	374	<0.001	<0.001	0.67	N/A
Leuk./lymph	2	190	<0.001	<0.001	0.65	<0.001
Liver	10	1424	<0.001	<0.001	0.95	<0.001
Lung	11	1769	<0.001	<0.001	1	1
Medulloblastoma	2	45	<0.001	<0.001	1	N/A
Melanoma	8	419	<0.001	<0.001	0.95	<0.001
Mesothelioma	6	663	<0.001	<0.001	0.96	<0.001
Myeloma	6	431	<0.001	<0.001	1	1
Non-mel.	1	53	<0.001	<0.001	-1	N/A
Oral	4	312	<0.001	<0.001	0.80	<0.001
Ovarian	19	2410	<0.001	<0.001	0.70	<0.001
Pancreatic	3	259	<0.001	<0.001	1	1
Pilomatricoma	1	7	0.008	0.014306	1	N/A
Prostate	11	909	<0.001	<0.001	0.71	<0.001
Renal	3	231	0.044	0.044181	0.13	N/A
Soft tissue sarcoma	1	30	<0.001	<0.001	1	N/A
Teratoid	3	57	<0.001	<0.001	1	N/A
Thyroid	7	182	<0.001	<0.001	1	N/A
vHL	1	46	<0.001	<0.001	1	N/A

N/A indicates that there were only two outcomes, and a non-linear fit is not measurable.

the categorical meta-analysis, but not Oncomine found Osteopontin levels to be also associated with melanoma, bladder, brain, and pancreatic cancer. This may reflect a gain in sensitivity by data ranking.

We asked whether the value of Osteopontin as a biomarker for cancer may be related to specific histological subtypes. According to Oncomine, Osteopontin levels were not significantly associated with FAB type of acute myelogenous leukemia (AML) (Fig. 2A). Similarly, Osteopontin expression was independent of breast cancer subtypes classified by receptor status (Fig. 2B). However, a p-value of 0.066 for overexpression of Osteopontin in triple-negative breast cancers suggested that an increase in the power of analysis may corroborate Osteopontin as a suitable marker for this

 Continued.

C, Cancer type	Oncomine (Over) p-value	Oncomine (Under) p-value	n
All	**0.050**	0.277	7385
Liver	**0.000**	1.000	176
Breast	**0.000**	0.714	225
Colorectal	**0.001**	0.968	428
Esophageal	**0.002**	0.998	13
Lung	**0.003**	0.926	405
Cervical	**0.012**	0.985	45
Ovarian	**0.016**	0.983	232
Sarcoma	**0.020**	0.980	51
Head and neck	**0.022**	0.995	244
Prostate	**0.043**	0.888	620
Melanoma	0.166	1.000	176
Brain	0.169	0.436	420
Pancreas	0.197	0.810	81
Bladder	0.592	0.684	245
Leukemia	0.895	0.242	1231
Renal	**0.032**	**0.005**	386

A, Evaluation of Osteopontin as a biomarker for cancer on the protein and RNA levels. The Pearson χ^2 test for independence assesses whether the Osteopontin ranks are independent among groups. The Mantel-Haenszel χ^2 test addresses the hypothesis that the ranking of a particular clinical variable within a study is linearly related to the Osteopontin level. B, Analysis of the categorized levels of Osteopontin compared against cancer status (cancer, pre-cancer, non-cancer disease and normal). Osteopontin levels and cancer were found to be dependent for all cancers combined ($p<0.001$), as well as in 31 individual cancers compared to normal controls with $p<0.05$ in all cases. In two cancers (non-melanoma skin cancer, gestational trophoblastic tumor), Osteopontin levels were significantly lower in the tumors than in the healthy controls. Of note, in skin cancer and gestational trophoblastic tumors, Osteopontin levels are also inversely correlated to cancer progression (2), suggesting a unique role for Osteopontin in these malignancies. Ampullary, ampullary neoplasm; gest. troph., gestational trophoblastic tumor; gran. cell, granular cell tumor; leuk./lymph, leukemia/lymphoma; non-mel., non-melanoma skin cancer; vHL, von Hippel-Lindau disease. C, Entries in Oncomine were analyzed for Spp1 (Osteopontin) as a biomarker for individual cancers and all cancers combined. The meta-analysis function in Oncomine calculated separate p-values for overexpression and for under-expression.

breast cancer subtype. This possibility would be consistent with a previous report that found high levels of an Osteopontin splice variant in breast cancers that were negative for ER, PR and Her2/Neu (13).

Osteopontin in primary tumors vs. metastases. We analyzed differences between the Osteopontin levels in primary tumors and metastases according to the published literature. For all cancers tested, the p-values were significant, but the correlation coefficients were negative in liver, bone and gastric cancers. Osteopontin was elevated compared to the primary tumor in the metastases of colorectal cancer, lung cancer, cervical cancer, breast cancer, melanoma and prostate cancer. In ovarian cancer, there was no change in the abundance of Osteopontin between primary tumors and metastases. For three malignancies, liver, bone and gastric cancer, Osteopontin levels in the metastases were lower than in the primary tumors (Table IIA).

In Oncomine, Osteopontin mRNA levels were higher in metastatic growths than in the primary tumors in gastrointestinal cancer, kidney cancer, melanoma, liver cancer, pancreatic cancer and lung cancer. In one of three studies, Osteopontin levels in breast cancer metastases were reduced compared to the primary tumors. For all tumors combined, there was a statistically significant increase ($p=0.032$) in Osteopontin levels in metastatic over primary cancers (Table IIB). In sum, Osteopontin is elevated compared to the primary tumors in the metastases of colorectal cancers, lung cancers and melanomas, but not in ovarian cancer, with discrepant results between the literature and the Oncomine database on liver, prostate and breast cancers.

Osteopontin in cancer risk and etiology. Osteopontin has been measured in relation to various risk factors for developing cancer. For some of them, positive correlations have been identified. Serum Osteopontin levels distinguished persons with exposure to asbestos who did not have cancer from those with exposure to asbestos who had pleural mesothelioma (14). Osteopontin overexpression in tumor tissue was significantly associated with betel nut chewing in esophageal cancer (15). In contrast, Osteopontin levels were not statistically significantly associated with alcohol consumption in oral (16) or esophageal cancers (15).

Smoking is a risk factor for several types of cancer. In non-small cell lung cancer, the patients' smoking status was reported in one study to influence circulating Osteopontin levels (17), whereas another study did not detect statistically significant differences in Osteopontin RNA levels, measured in cancer tissues from smokers vs. non-smokers (18). Further, Osteopontin levels were not statistically significantly associated with smoking in oral (16) or esophageal cancers (15). This was also reflected in Oncomine, where smoking was not associated with overexpression ($p=0.297$) or underexpression ($p=0.412$) of Osteopontin (Fig. 3).

In most cancers, Osteopontin is not indicative of the underlying mechanism of transformation. Osteopontin levels did not differentiate melanoma in sun-exposed vs. non-sun-exposed areas (19). The abundance of Osteopontin in liver cancer was independent of whether cirrhosis was present or not (20-23). Neither did Osteopontin distinguish *B-raf* wild-type from *B-raf* mutant thyroid cancers (24). In multiple myeloma, by contrast, high Osteopontin expression inversely correlated with bone disease and was significantly up-regulated in patients with *maf* translocations, particularly in the fraction lacking bone disease (25).

Osteopontin is a TH_1 cytokine that plays important roles in anti-viral host defenses. Consistently, in cancers of viral origin the underlying etiology correlates with Osteopontin levels. Hepatocellular carcinoma may be caused by viral

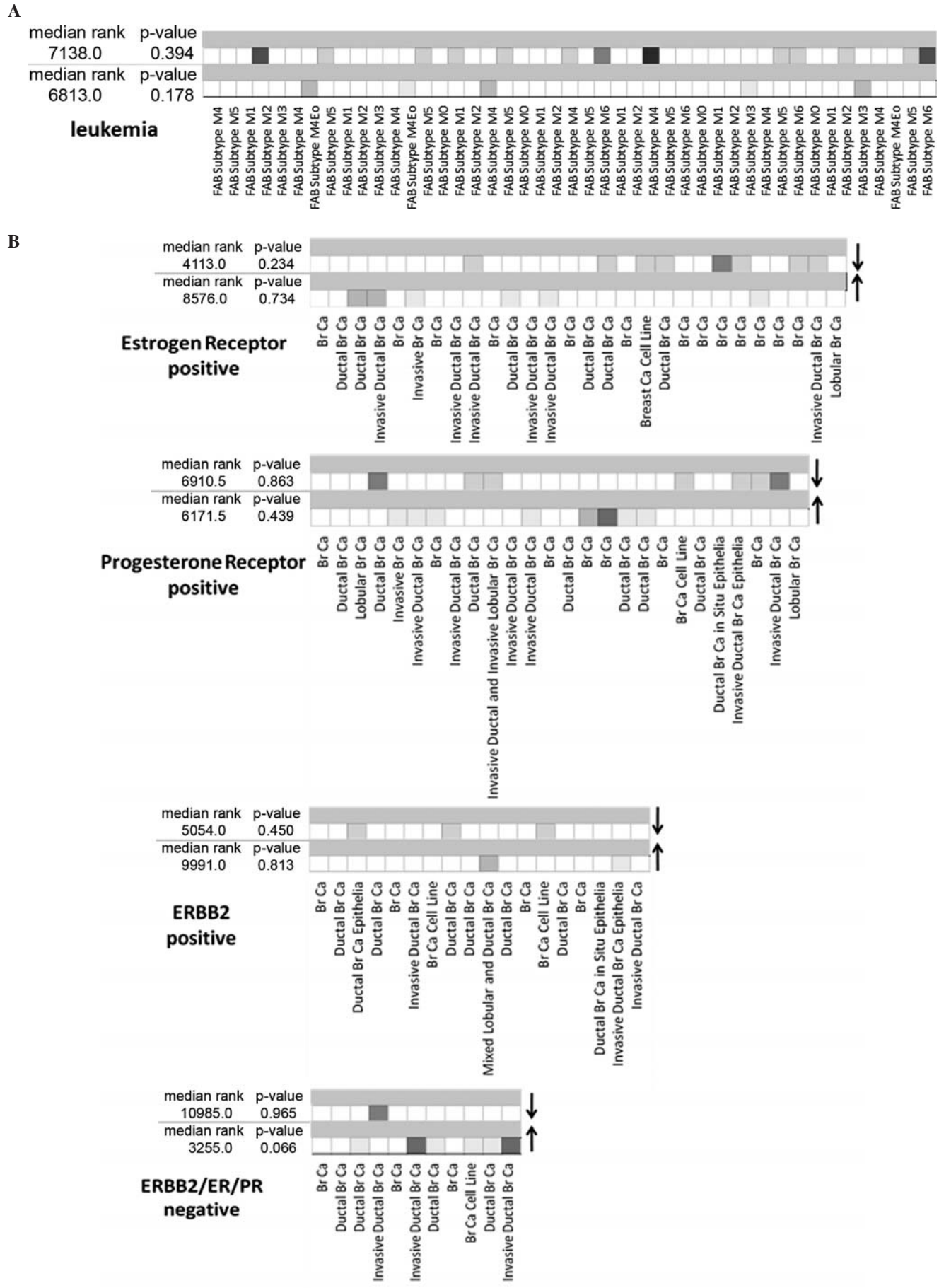

Figure 2. Osteopontin and cancer type in Oncomine. (A) Leukemia. The results reflect the evaluation of 8 data sets containing 1117 samples. In data sets in the upper row, Osteopontin is under-expressed; in data sets in the lower row, Osteopontin is over-expressed. The median ranks and p-values are calculated separately for over- and underexpressions. (B) Breast cancer. The analysis is broken down into ER positive, PR positive, ERBB2 positive and triple negative cancers.

Osteopontin levels in primary vs. metastatic cancers.

A,	Studies	Patients	Pearson χ^2	Pearson p-value	Linear χ^2	Linear p-value	Correlation coefficient
All cancers	27	2744	1751.78	<0.001	1751.14	<0.001	0.80
Lung	2	1571	1571.00	<0.001	1570.00	<0.001	1.00
Colorectal	6	390	390.00	<0.001	389.00	<0.001	1.00
Breast	5	279	93.32	<0.001	92.99	<0.001	0.58
Gastric	2	85	85.00	<0.001	84.00	<0.001	-1.00
Cervical	1	80	80.00	<0.001	79.00	<0.001	1.00
Melanoma	3	78	78.00	<0.001	77.00	<0.001	1.00
Prostate	2	46	46.00	<0.001	45.00	<0.001	1.00
Bone	2	59	35.26	<0.001	34.66	<0.001	-0.77
Liver	1	30	30.00	<0.001	29.00	<0.001	-1.00
Ovarian	3	126	9.17	0.002	9.10	0.003	0.27

			Oncomine		
B,	Literature p-value	n	(Over) p-value	(Under) p-value	n
All cancers	**0.000**	2744	**0.032**	0.739	3576
Colorectal	**0.000**	390	**0.000**	1.000	118
Lung	**0.000**	1571	**0.032**	0.323	21
Cervical	**0.000**	80			
Breast	**0.001**	279	0.987	0.353	20
Melanoma	**0.004**	78	**0.005**	1.000	147
Prostate	**0.018**	46	0.539	0.989	6
Kidney			**0.000**		9
Pancreas			**0.024**		6
Head and neck			0.092	0.860	22
Ovarian	0.228	126	0.684	0.266	116
Sarcoma			0.763	0.601	41
Liver	**0.031 (anti)**	30	**0.006**		6
Bone	**0.016 (anti)**	59			
Gastric	**0.000 (anti)**	85			

Osteopontin in primary vs. metastatic tumors. A, Data from the literature compared to microarray data from Oncomine. As a test for independence of the ranked data we utilized the Pearson χ^2 test. To assess linear and non-linear trends of the ranked data we applied the Mantel-Haenszel χ^2 test. The published results had lower Osteopontin in 1510 primary tumors and 106 metastatic tumors, higher Osteopontin in 160 primary tumors and 968 metastatic tumors. B, Oncomine calculates separate p-values for Osteopontin overexpression and Osteopontin underexpression. P-values in bold are considered significant. Negative correlation coefficients indicate inverse correlation.

infections. Categorized Osteopontin levels (RNA and protein combined) inversely correlate with the presence of hepatitis B virus (HBV) or hepatitis C virus (HCV) (Table III). High serum Osteopontin levels are associated with B-cell non-Hodgkin lymphoma and HCV infection (26). Polymorphisms in the Osteopontin gene, *spp1*, are associated with HBV clearance and occurrence of hepatocellular carcinoma. An allele that is associated with poor viral clearance also correlates with an early age of onset of hepatocellular carcinoma (27). For bacterial pathogens the TH_1 effects of Osteopontin are less relevant. In gastric cancer, Osteopontin levels were not different between *H. pylori* positive and *H. pylori* negative patient groups (15).

Discussion

Osteopontin has been associated with tumor progression and metastasis. Numerous publications have tested it as a biomarker. However, these diverse clinical studies have not yet been analyzed in their entirety. Neither has Osteopontin

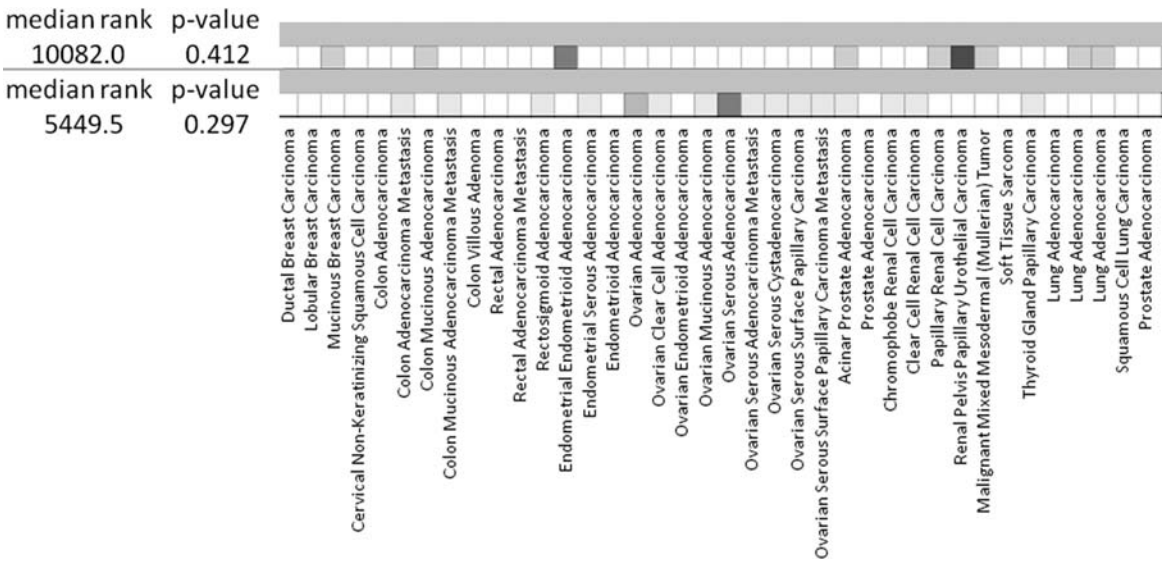

Figure 3. Osteopontin and smoking in cancer. Osteopontin levels in cancer patients who are smokers vs. non-smokers according to Oncomine. The results represent 14 data sets with 2433 samples.

Table III. Osteopontin and etiology of liver cancer in the literature.

	Studies	Patients	Pearson p-value	Linear p-value	Correlation coefficient
Hepatitis B virus	4	755	<0.001	<0.001	-0.73
Hepatitis C virus	2	325	<0.001	<0.001	-1

As a test for independence of the ranked data we utilized the Pearson χ^2 test. To assess linear trends of the ranked data we applied the Mantel-Haenszel χ^2 test.

found entry as a cancer marker into routine diagnostics. Meta-analysis has been a valuable tool in biomarker validation. Here we report results of a meta-analysis with data from two distinct sources, the published literature and microarray data deposited in Oncomine. Rather than combining the data sets, we evaluated the literature data with the novel approach of categorical meta-analysis, and we studied the Oncomine data with the meta-analysis routines included in the software.

One of the major limitations of meta-analysis is the detection of true signals over the noise derived from heterogeneous input data. Categorical data analysis has a self-normalizing effect on study-to-study variations and may therefore be superior to conventional meta-regression algorithms. For the evaluation of Osteopontin as a biomarker for cancer, we have found conventional and categorical meta-analysis to be in agreement. This was not the case for the correlation of Osteopontin levels with tumor grade and stage. Here, the improved sensitivity of the categorical analysis is required to detect the existing trends in the published data sets (2). For the correlation of Osteopontin with several clinical readouts there are discrepancies, which will require further research to obtain clarification.

The interest in Osteopontin as a biomarker has been focused mostly on changes in its protein or RNA levels. The measurements of Osteopontin abundance have also been the focus of this meta-analysis. However, Osteopontin is subject to abundant modifications on the genetic and epigenetic levels, some of which are also relevant for carcinogenesis. Polymorphisms in the Osteopontin gene, *spp1*, have recently been associated with hepatocellular carcinoma (27) and with lung cancer (17). Gene amplification of *spp1* has been studied in hepatocellular, ovarian and endometrial cancers (28,29). Osteopontin is subject to alternative splicing. Recent investigations have demonstrated the potential benefit in measuring only the shortest of three Osteopontin splice variants, Osteopontin-c, in cancer diagnostics (13,30). In contrast to the full-length form, Osteopontin-a, the shortest splice variant has not been found in untransformed cells or tissues. It is therefore more cancer-specific than total Osteopontin. Fragments of the Osteopontin protein excreted into the urine have been used to diagnose ovarian cancer (31) and renal conditions (32).

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