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Original Article

Role of adenylate cyclase-associated protein 1 in cancers

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Abstract: Cancer is a leading cause of death worldwide. Adenylate cyclase-associated protein 1 (CAP1) may play a role in cell motility and in the development of certain types of cancer. Here, we explore the expression of CAP1 in primary tumor tissues using the Oncomine database for four cancers with the highest mortality. In addition, we studied the correlation between CAP1 expression and overall survival using Kaplan-Meier analysis. We analyzed the structure and function of CAP1 in cancers. We found widespread upregulation of the expression of CAP1 gene in primary tumor tissues. There was also a correlation between CAP1 expression levels and patient survival. These data provide additional evidence for CAP1 as a biomarker for cancer studies and as a target for cancer diagnose.

Keywords: CAP1, cancer, overall survival, actin cytoskeleton

Introduction

Cancer remains a leading cause of morbidity and mortality worldwide, and the cancer-related morbidity and mortality rate is expected to increase in the next few decades. According to the World Health Organization, if the global cancer rates remain unchanged, by 2030, the number of new cancer cases will reach 21.4 million [1]. Lung cancer is believed to be the most common fatal neoplastic disease in the world today [2]. Breast cancer ranks second as the cause of cancer death in women (after lung cancer) [3]. Epithelial ovarian cancer is also a leading cause of cancer-related deaths in women and the most lethal gynecological malignancy [4]. Gastric cancer (GC) is the third most common cause of cancer-related death in the world, which makes it a major global health issue [5]. These leading types of cancers were the focus of our analysis.

The *CAP* gene (also called *SRV2*) was isolated in *Saccharomyces cerevisiae* as a suppressor of activated RAS [6]. CAPs are present in a wide range of organisms, from yeast to mammals. Mammals have two CAP isoforms-CAP1 and CAP2. The same isoforms in different mammalian species share extremely high homology, whereas the homology between two isoforms from the same species is relatively low [6, 7]. CAP1 is ubiquitously expressed in almost all tissues and cells, whereas CAP2 has a more restricted expression pattern and is found predominantly in skeletal muscle, cardiac muscle, brain, and skin [7, 8]. All the studies conducted so far indicate that CAP1 is required in most cells, while CAP2 appears to have unique roles in specific cells or tissues. Most studies so far have investigated CAP1. Therefore, in this study too, we have explored the relationship between CAP1 expression and four of the most prevalent types of cancer.

Structural analysis of CAP

The structure of CAP can only be speculated due to a lack of X-ray crystallographic and nuclear magnetic resonance (NMR) spectroscopy data [6]. The major structural and functional domains of CAP are illustrated in **Figure 1**. Homologues of CAP in humans were identified in the early 1990s. CAP homologues have three conserved structural domains-the N-ter-

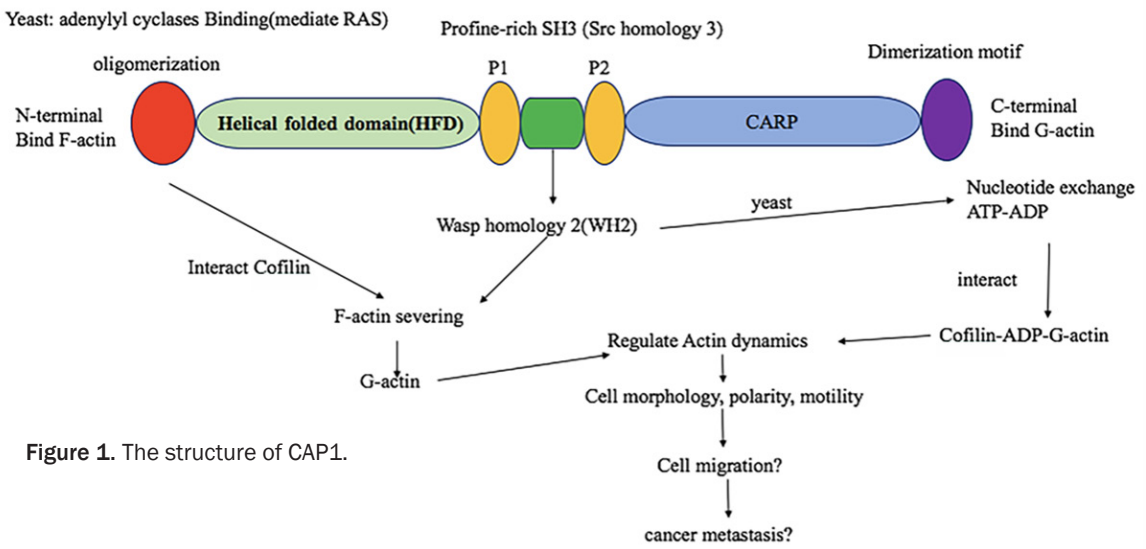


Figure 1. The structure of CAP1.

minal domain, the C-terminal domain and a proline-rich middle domain [6, 9]. All three domains contribute to actin filament turnover through interactions with cofilin, and G- and F-actin [9]. In mammals, the C-terminal domain binds and sequesters G-actin, and also catalyzes nucleotide exchange of ATP onto ADP-bound G-actin, whereas in yeast, this function is further enhanced by the Wasp homology 2 (WH2) domain, which is located near the C-terminus of the middle domain [10, 11]. Nucleotide recharging on ADP-G-actin in complex with cofilin is a key rate-limiting step, and CAPs relieve the inhibitory effect of cofilin on recharging. The N-terminal domain of CAP binds the cofilin-ADP-G-actin complex first for subsequent nucleotide exchange, and CAPs can also directly bind F-actin to promote its severing [10]. Therefore, CAP1 regulates both actin filaments and may play a role in cell motility and in the development of certain types of cancer.

Functional analysis of CAP

CAP was first identified as a component of the yeast adenylyl cyclase complex and independently in genetic screenings to identify components of the yeast Ras/cAMP signaling pathway. The CAP protein shares high homology with the *Saccharomyces cerevisiae* CAP protein, which is involved in the cyclic AMP pathway. Human CAP can interact with cofilin and actin. Alternatively, spliced transcript variants have been identified [6].

Mouse CAP1 is phosphorylated, which suggests that the activity and localization of CAP can be regulated through kinase signaling pathways. CAP1 is also an intracellular substrate of a matrix metalloproteinase, which indicates that the protein level of CAP is controlled by proteolysis. CAP has many functions, such as actin binding, adenylyl cyclase association in yeast, SH3 binding, oligomerization, cAMP signaling, kinase signaling, vesicle trafficking, sarcomere organization, neuronal growth, apoptosis, and organ/tissue development [12]. CAP has been implicated in both cell migration and cytokinesis, and cell migration and cytokinesis are known to be aberrantly regulated in cancer cells.

Therefore, we extracted data from the database Oncomine for lung and bronchus, kidney, breast, gastric and liver, ovarian and pancreatic cancers, focusing on clinical specimens of cancer vs. normal patient datasets, and separated by subtype when possible (**Figure 2**). We also tested for the effect of CAP1 expression on patient overall survival using Kaplan-Meier Plotter. Here we present a summary of the results we obtained (**Figure 2**).

Material and methods

Oncomine analysis

The expression level of CAP1 genes in the selected cancers was analyzed using Oncomine

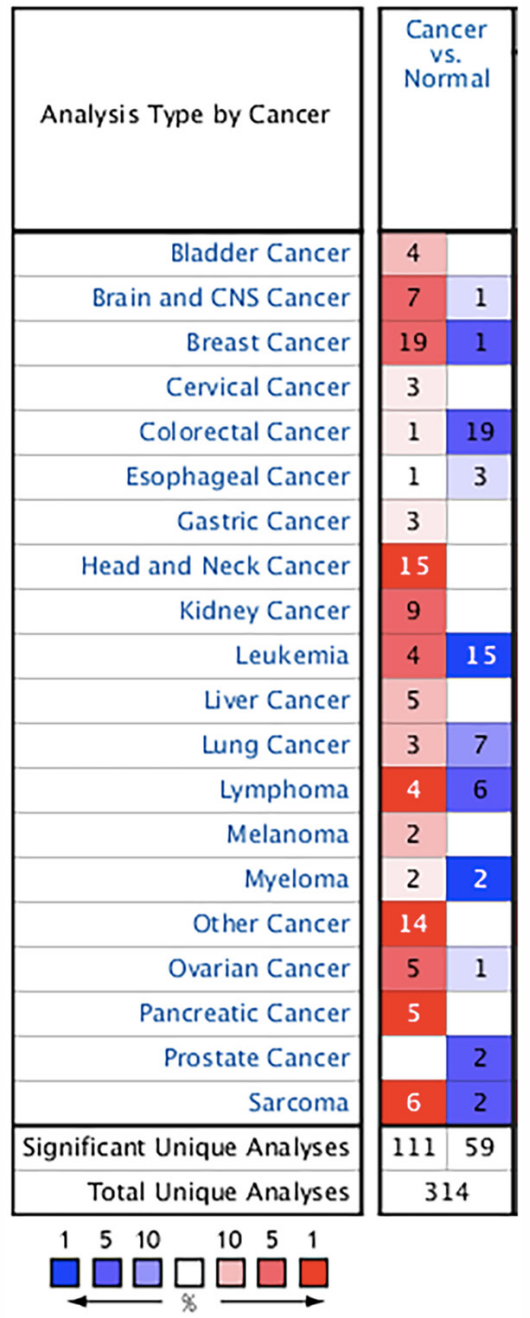


Figure 2. CAP1 mRNA expression in different tumor types. This graph compares the number of datasets that had significant mRNA overexpression (left column, red) and underexpression (right column, blue) of the specified gene in cancer versus normal tissue. The datasets were obtained with the following parameters: cell color is determined by the best gene rank percentile for the analyses within the cell; *P*-value threshold of 0.01.

[13]. For this, we compared clinical specimens of cancer vs. normal patient datasets. In order to reduce our false discovery rate, we selected $P < 0.01$ as a threshold. We analyzed the results for their *P*-values, fold change, and cancer subtype.

Kaplan-Meier plotter analysis

The prognostic value of the CAP1 genes in ovarian, breast and gastric cancer was analyzed using Kaplan-Meier Plotter (http://kmplot.com/analysis/), a database that integrates gene expression data and clinical data [14]. To date, Kaplan-Meier Plotter contains information on 22,277 genes and their effect on survival in 1,117 breast, 1,583 ovarian and 876 gastric cancer patients. We focused our analysis on overall survival patient information. The patient samples have been split into two groups. The two patient groups (higher and lower expression levels) were compared using a Kaplan-Meier survival plot. The hazard ratio with 95% confidence intervals and log rank *P* value was calculated. We analyzed the best specific probes (JetSet probes) that recognized CAP1. In order to reduce our false discovery rate, we selected $P < 0.01$ as a threshold.

Statistical analysis

Survival curves generated by the Kaplan-Meier plots. All results are displayed with *P* values from a log-rank test. Similarly, with Oncomine. Statistical significance of the data (*P*-values) was provided by the program.

Results: association of CAP with different types of cancer

Lung cancer

Lung cancer ranks first with regard to both its prevalence and mortality rates among all types of cancer. Approximately 85% of lung cancer cases are non-small cell lung cancer (NSCLC), which includes adenocarcinoma (AD), squamous cell carcinoma (SCC), large cell carcinoma and bronchioalveolar carcinoma. NSCLC is a leading cause of cancer-related death. Despite extensive research and clinical efforts, the prognosis of NSCLC remains poor: the 5-year survival of patients with metastatic NSCLC is < 10% [15-17].

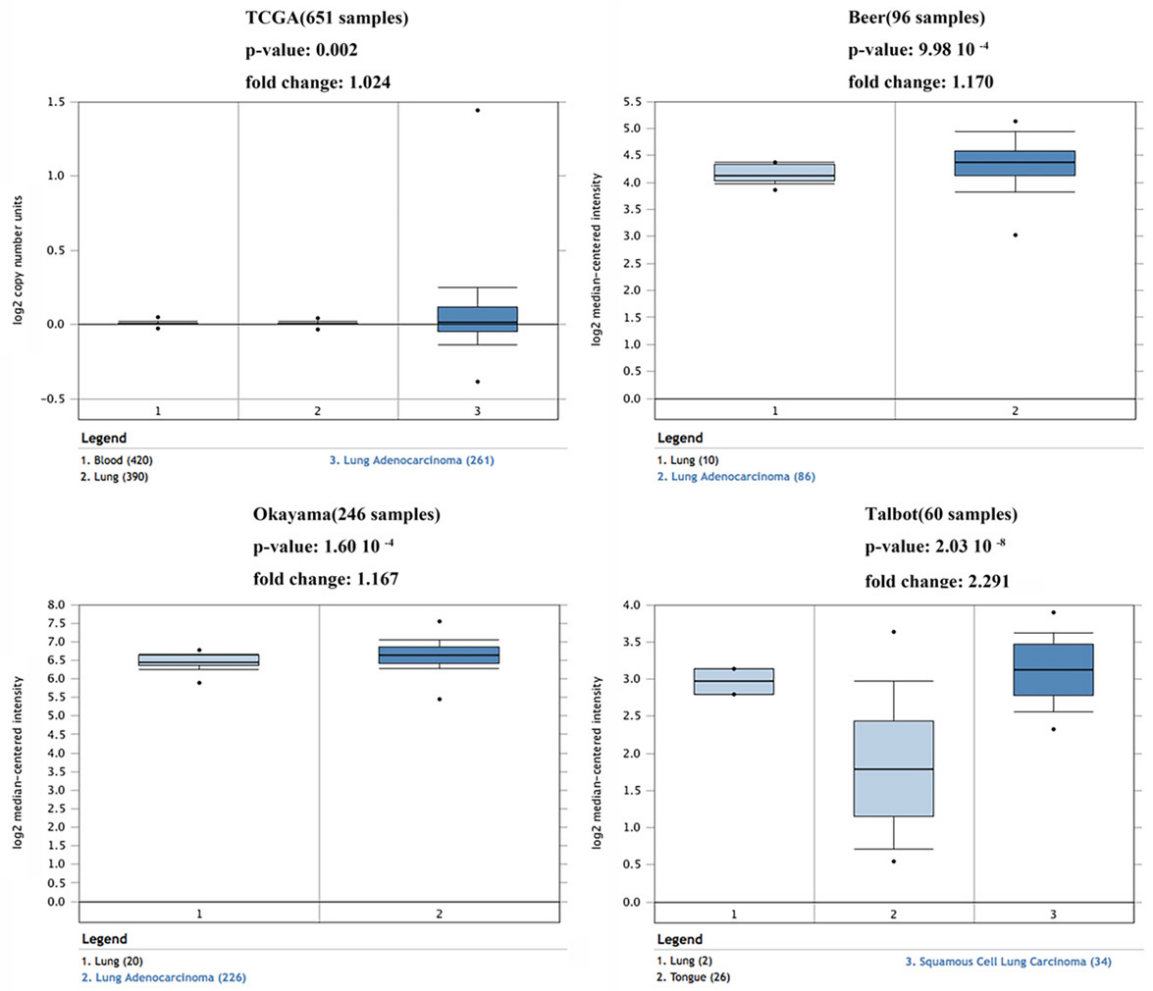
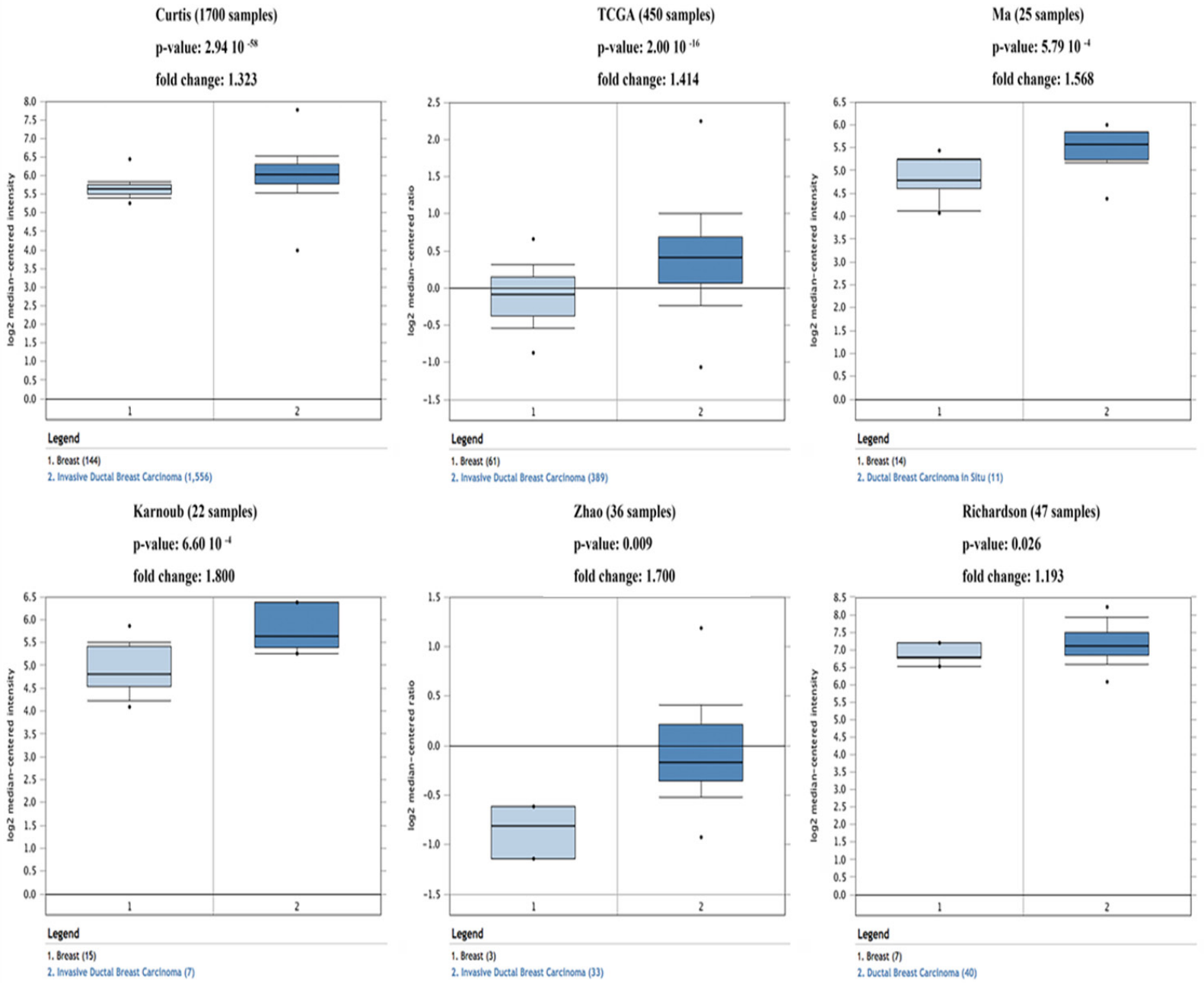


Figure 3. CAP1 gene analysis in lung cancer (Oncomine database).

We analyzed cDNA microarray data to determine whether CAP1 genes were differentially expressed in human lung cancer compared to normal lung tissue. The Oncomine database contains four microarray datasets [18-21] that compare gene expression levels in 607 lung cancer samples and 422 normal lung tissue samples (http://www.oncomine.org). Data were retrieved by using the search terms “CAP1”, “NSCLC” and “Cancer vs. Normal Analysis”. The literature retrieved from the search indicated that the expression of CAP1 was significantly higher in lung neoplastic tissues than in control specimens. Overexpression of CAP1 was found in squamous cell carcinoma and adenocarcinoma (**Figure 3**). These results are similar to our previous studies. In our previous study [22], 24 lung cancer patients and 6 control subjects with non-neoplastic lung condition(s) who underwent resection of neoplastic and non-neoplastic lung lesions were recruited from our hospital. Then, we performed real-time PCR, western blot analysis, and immunohistochemistry to analyze the relative levels of CAP1 mRNA and protein in biopsy specimens. In addition, multivariate regression analysis was performed to determine the correlation of the immunohistochemical CAP1 signal with cancer type and stage.

The results demonstrated that the expression of CAP1 was significantly higher in neoplastic tissues than in control specimens. In our previous study [22], 83 lung cancer patients who underwent surgical resection of neoplastic and non-neoplastic lung lesions were recruited from our hospital. In the CAP1-positive group, the 3-year survival rate was 0.143, and


Curtis (1700 samples)
p-value: 2.94 10 -58
fold change: 1.323
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1. Breast (144)
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TCGA (450 samples)
p-value: 2.00 10 -16
fold change: 1.414
log2 median-centered ratio
Legend
1. Breast (61)
2. Invasive Ductal Breast Carcinoma (389)
Ma (25 samples)
p-value: 5.79 10 -4
fold change: 1.568
log2 median-centered intensity
Legend
1. Breast (14)
2. Ductal Breast Carcinoma in Situ (11)
Karnoub (22 samples)
p-value: 6.60 10 -4
fold change: 1.800
log2 median-centered intensity
Legend
1. Breast (15)
2. Invasive Ductal Breast Carcinoma (7)
Zhao (36 samples)
p-value: 0.009
fold change: 1.700
log2 median-centered ratio
Legend
1. Breast (3)
2. Invasive Ductal Breast Carcinoma (33)
Richardson (47 samples)
p-value: 0.026
fold change: 1.193
log2 median-centered intensity
Legend
1. Breast (7)
2. Ductal Breast Carcinoma (40)

Figure 4. CAP1 gene analysis in brest cancer (Oncomine database).

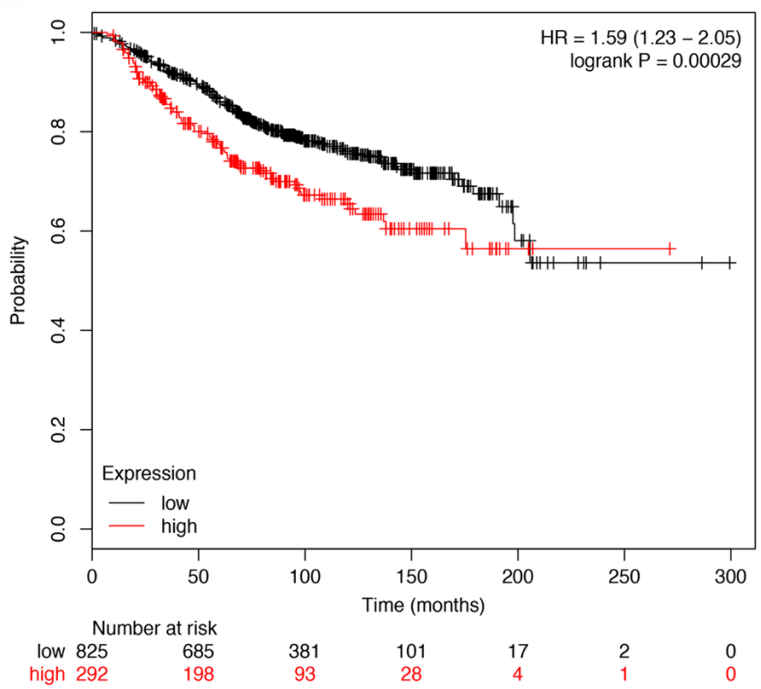

Figure 5. CAP1 genes in (Kaplan-Meier Plotter). Kaplan-Meier plots showing overall survival in breast cancer. In red: patients with expression above the median and in black, patients with expressions below the median.

the rate was 0.377 in the group that showed low expression of CAP1 (P = 0.045, log-rank test). Therefore, a high level of CAP1 expression might indicate poor prognosis of lung cancer.

Breast cancer

Breast cancer ranks second as the cause of cancer-related death in women (after lung cancer) [3]. Breast cancer is the most frequently diagnosed neoplastic disease in women around menopause, often leading to a significant reduction in these women's ability to function normally in everyday life [23]. Each year, 2,300 new cases of breast cancer are diagnosed in men, and about 230,000 new cases are diagnosed in women. Invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC) are the major histological types of invasive breast cancer among women of different races worldwide, with an incidence ranging from 47-79% to 2-15%, respectively. It is not clear whether IDC and ILC represent molecularly distinct entities and what genes might be involved in the development of these two phenotypes [24]. The overall 5-year survival rate is 89.2% in localized or low-grade breast cancer [23].

Overall, the Oncomine database contains six microarray datasets [21, 25-29] that compare gene expression levels in 2018 breast cancer and 244 normal samples (http://www.oncomine.org). Data were retrieved by using the search terms "CAP1", "breast cancer" and "Cancer *vs*. Normal Analysis". The Oncomine analysis showed significant levels of CAP1 overexpression in most breast cancer types, especially IDC (**Figure 4**). Kaplan-Meier analysis revealed that high levels of CAP1 expression correlate with lower survival rates (**Figure 5**). In addition, it has been found that overexpression of CAP1 genes can promote epithelial to mesenchymal transition in cell lines [30, 31]. These studies emphasize the importance of analysis of the expression of CAP1 genes during cancer progression.

Ovarian cancer

Ovarian cancer is the seventh most common cancer in women worldwide, with 239 000 new cases diagnosed in 2012 [32]. Ovarian cancer refers to a diverse set of histological types of cancers. Most ovarian cancers are epithelial in origin, with high-grade serous carcinomas accounting for 70%-80% of cases; the rarer types include clear cell (3%), endometrioid (< 5%), and mucinous (< 3%) cancer [33]. The prognosis of ovarian cancer is usually poor, due to the lack of either specific symptoms or effective screening and diagnostic methods in identifying early-stage disease. As a result, over 70% of patients are diagnosed only in the advanced stage of the disease, which makes the 5-year survival rate only 30%-44% [34].

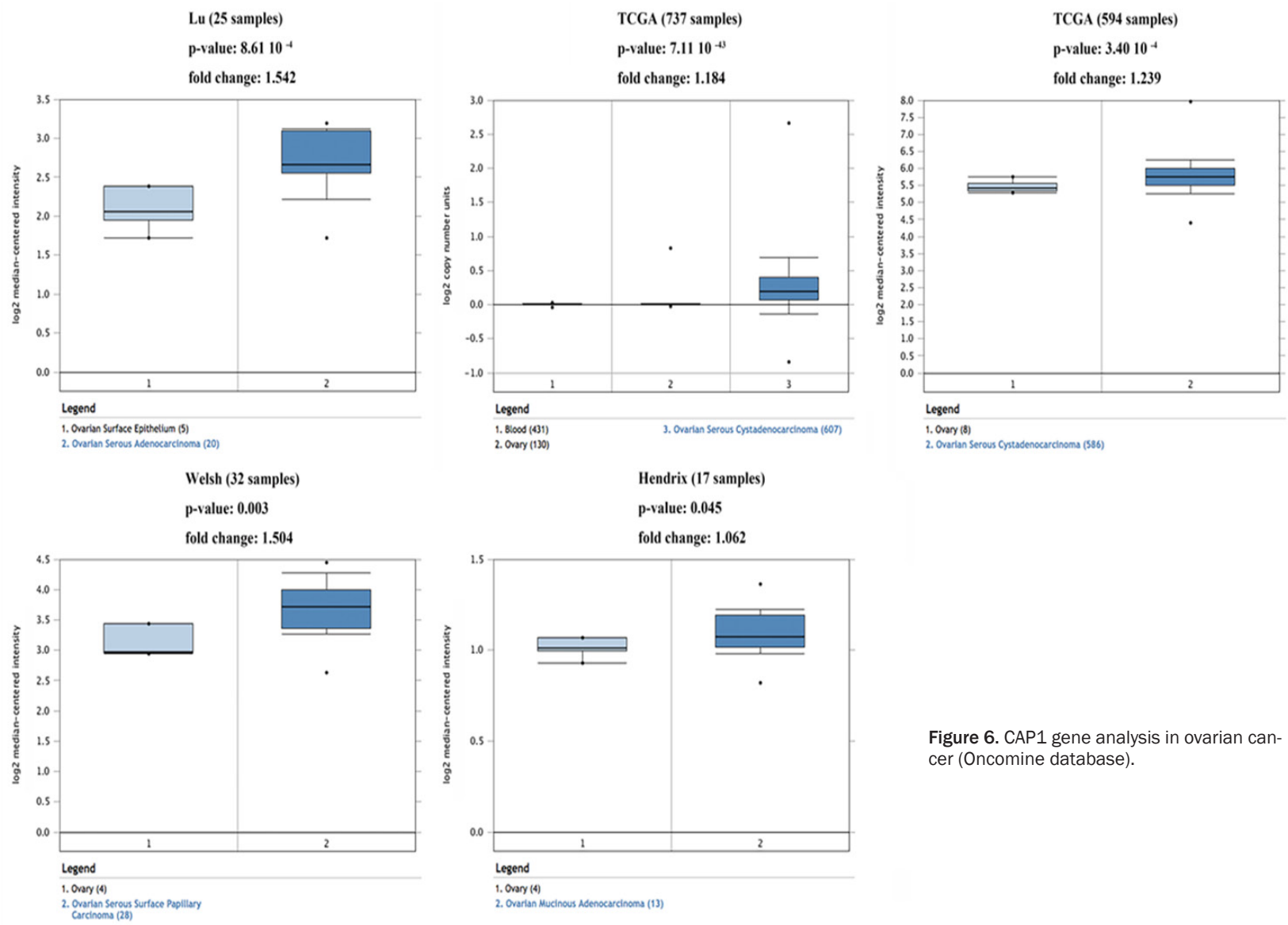

Figure 6. CAP1 gene analysis in ovarian cancer (Oncomine database).

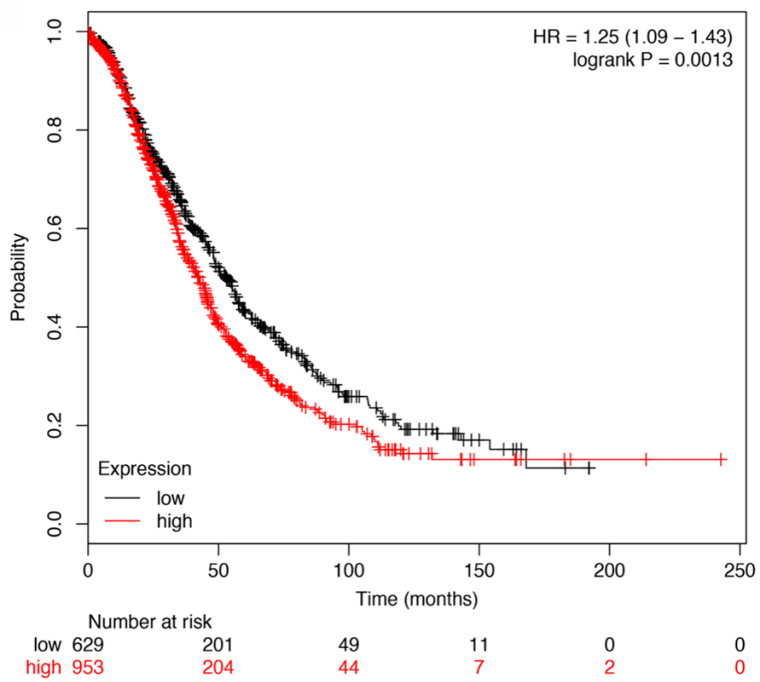

Figure 7. CAP1 genes in (Kaplan-Meier Plotter). Kaplan-Meier plots showing overall survival in ovarian cancer. In red: patients with expression above the median and in black, patients with expressions below the median.

The Oncomine database contains five microarray datasets [21, 35-37] that compare gene expression levels in 1241 ovarian cancer samples and 147 normal samples (http://www.oncomine.org). Data were retrieved using the search terms "CAP1", "ovarian cancer" and "Cancer vs. Normal Analysis". Oncomine analysis of neoplastic vs. normal tissue showed that CAP1 expression was significantly upregulated in several ovarian cancer types (**Figure 6**). Kaplan-Meier analysis revealed that high levels of CAP1 expression were correlated with lower patient survival rates (**Figure 7**).

Gastric cancer

Stomach cancer, also called *gastric cancer*, is a type of cancer that originates in the stomach. About 90% to 95% of cancers of the stomach are adenocarcinomas. Gastric cancer (GC), the third leading cause of global cancer death, is a malignant disease with a high mortality rate despite its declining incidence in the recent decade [38, 39]. Multimodal treatment strategies including surgery, chemotherapy and radiotherapy can improve local and regional tumor control and decrease the rate of systemic metastasis [40, 41]. However, the overall prognosis of advanced-stage disease remains poor. The overall 5-year relative survival rate of individuals with stomach cancer in the United States is 29%. One reason the overall survival rate is poor in the United States is that most stomach cancers are diagnosed at an advanced rather than an early stage. The stage of the cancer has a major effect on a patient's prognosis.

The Oncomine database contains three microarray datasets [42-44] that compare gene expression levels in 80 gastric cancer samples and 132 normal samples (http://www.oncomine.org). Data were retrieved by using the search terms "CAP1", "gastric cancer" and "Cancer vs. Normal Analysis". Oncomine analysis of neoplastic *vs.* normal tissue showed that CAP1 were significantly upregulated in several gastric cancer types (**Figure 8**). Kaplan-Meier analysis revealed that high levels of expression of CAP1 were correlated with lower patient survival rates (**Figure 9**).

Discussion

Firstly, in our analysis of Oncomine we found that CAP1 was over-expression in various cancer types compared with normal tissues. Secondly, The Kaplan-Meier plots predict that in the case of some cancers, a high level of CAP1 expression is associated with poor prognosis. These results indicate that high expression of CAP1 was associated with poor prognosis in cancers. Therefore, if a functional correlation between CAP levels and cancer phenotypes can be established, CAP might serve as a diagnostic marker or therapeutic target for certain types of cancer.

We also analyzed the role of CAP1 in other cancers (**Table 1**). Oncomine analysis revealed that CAP1 was significantly in Brain and CNS

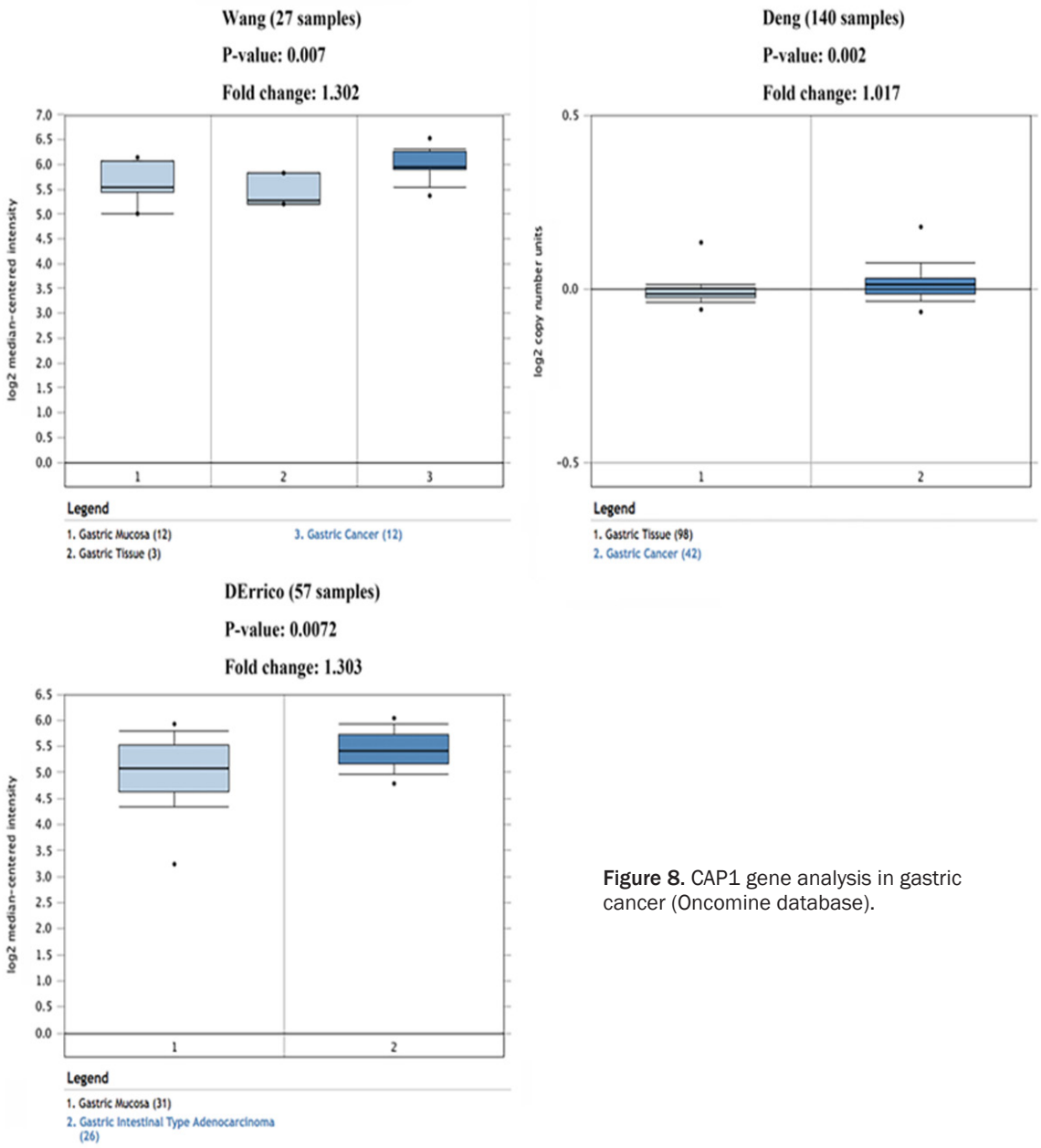

Figure 8. CAP1 gene analysis in gastric cancer (Oncomine database).

Cancer [21, 45-48], Head and Neck Cancer [18, 49-55], pancreatic cancer [56-59], Liver Cancer [60-62], kidney cancer [63-67]. The results were similar with the above results. Those results further curtained that CAP1 was over-expression in cancer.

Although CAP proteins have been studied for more than a decade and are present in all organisms, many questions remain unanswered about the mechanisms underlying the functions of CAP [39]. Cell migration is driven by actin dynamics, which is the repeated cycling of monomeric actin (G-actin) into and out of filamentous actin (F-actin) [40]. CAP1 is a conserved actin-regulatory protein, which is implicated in cell motility and the invasiveness of human cancers. It works in synergy with another actin regulatory protein, cofilin, to accelerate actin dynamics. Hence, the knockdown of CAP1 has been shown to reduce cell motility and migration [41]. Given the critical role of actin filament reorganization in cell migration and the regulatory role of CAP1 in

Table 1. Changes of CAP1 gene expression in other cancer

P-value	Fold change	Rank (top%)	Dataset	Samples	Reference
Brain and CNS Cancer *vs*. Normal					
$1.81\ 10^{-4}$	1.66	2%	French	10	[45]
$7.51\ 10^{-4}$	1.701	14%	Shai	34	[46]
0.004	1.82	5%	Pomeroy	9	[47]
0.008	1.048	15%	Beroukhim	140	[48]
$8.23\ 10^{-10}$	1.374	5%	TCGA	552	[21]
$2.67\ 10^{-23}$	1.046	16%	TCGA2	619	[21]
Head and Neck Cancer *vs*. Normal					
$1.68\ 10^{-12}$	3.33	1%	Estilo	57	[49]
$1.37\ 10^{-11}$	3.095	2%	Talbot	47	[18]
$2.54\ 10^{-8}$	1.530	7%	Peng	79	[50]
$3.61\ 10^{-8}$	1.512	5%	Ginos	54	[51]
$4.3\ 10^{-7}$	2.439	2%	Pyeon	24	[52]
$3.58\ 10^{-5}$	1.099	4%	Giordano	30	[53]
0.003	1.191	8%	He	18	[54]
0.008	1.379	15%	Ye	38	[55]
Pancreatic Cancer *vs*. Normal					
$1.24\ 10^{-9}$	2.52	5%	Badea	78	[56]
$1.42\ 10^{-5}$	4.732	1%	Segara	17	[57]
$6.86\ 10^{-5}$	2.308	8%	Pei	52	[58]
$5.83\ 10^{-5}$	2.958	3%	Logsdon	27	[59]
Liver Cancer *vs*. Normal					
$2.07\ 10^{-27}$	1.227	8%	Roessler	445	[60]
$9.46\ 10^{-5}$	1.3	13%	Mas	115	[61]
0.002	1.345	18%	Wurmbach	75	[62]
Kidney Cancer *vs*. Normal					
$1.88\ 10^{-6}$	1.451	4%	Gumz	20	[63]
$7.17\ 10^{-10}$	1.774	7%	Jones	92	[64]
$7.12\ 10^{-8}$	1.429	4%	Beroukhim	70	[65]
$1.37\ 10^{-4}$	2.131	6%	Yusenko	67	[66]
0.003	1.416	9%	Lenburg	18	[67]

actin filament reorganization, it is logical to hypothesize that CAP1 may be associated with tumors.

The importance of our analysis on CAP1 gene expression on cancer development, and on cancer patient diagnosis, treatment and survival needs to be further evaluated. First, the correlation between transcript and protein upregulation for different tumor types needs to be researched, as it is protein activity that will be the target of therapy. Second, additional research on the *in vitro* and *in vivo* tumorigenic potential verify the understudied CAP1. All together, these studies could lead to cancer diagnostic and predictive tools, and help develop more effective and specific cancer treatments.

Acknowledgements

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Disclosure of conflict of interest

None.

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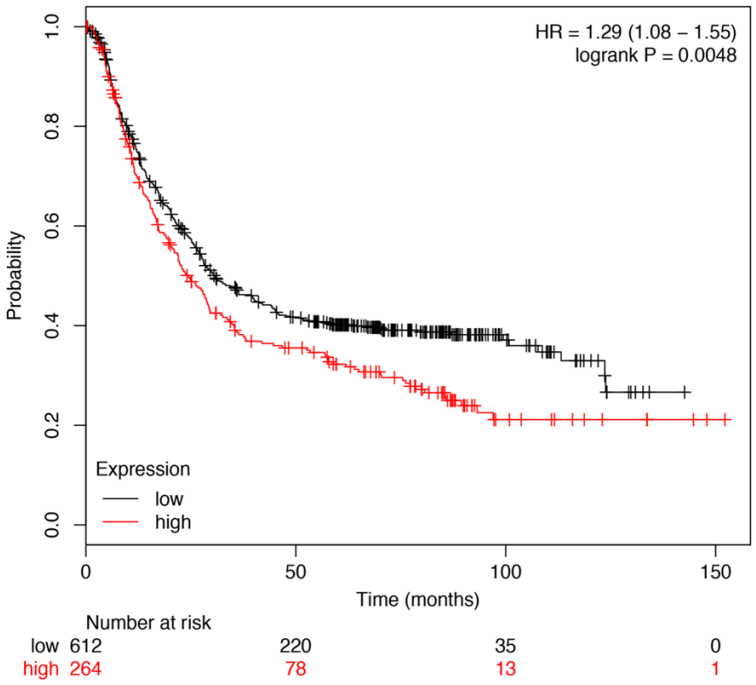

Figure 9. CAP1 genes in (Kaplan-Meier Plotter). Kaplan-Meier plots showing overall survival in gastric cancer. In red: patients with expression above the median and in black, patients with expressions below the median.

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