Nuclear TAZ expression associates with the triple-negative phenotype in breast cancer

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Abstract

The Hippo signaling pathway, a conserved regulator of organ size, has emerged as an important regulatory pathway in cancer. The final transducer effectors of this pathway in mammals are the oncoproteins TAZ and YAP1, which are transcriptional coactivators of target genes involved in cell proliferation and survival. TAZ has been previously reported to play a role in tumorigenesis in breast cancer, but detailed analyses of the different breast cancer phenotypes have not been conducted thus far. We analyzed TAZ expression by immunohistochemistry in a retrospective series of 640 invasive breast carcinomas, comprising estrogen/progesterone receptor-positive (ER+/PR+), HER2-positive, and triple-negative (TN) tumors. We found a strong association of TAZ nuclear expression with the TN phenotype (60.5% TAZ-positive, $P<0.001$), which was strengthened when stratified into the basal-like subtype (70.8% TAZ-positive, $P<0.001$). Moreover, 90% of metaplastic breast carcinomas with morphological epithelial–mesenchymal transition features were TAZ-positive. We also investigated whether amplification or differential DNA methylation of the *TAZ*-encoding locus could account for the observed enhanced TAZ protein expression in the TN/basal phenotype. Amplification of the *TAZ* locus was analyzed by fluorescence *in situ* hybridization in 30 TN tumors, and we found gene amplification in some cases (6.45%). DNA methylation analysis was performed using the Sequenom MassArray MALDI-TOF platform, and we observed similar low methylation levels both in TN ($n=25$) and ER+/PR+ ($n=26$) tumors. These results were further confirmed using a panel of breast cancer cell lines and using the TCGA dataset. Finally, patients with strong TAZ expression showed poorer clinical outcomes with respect to both recurrence and overall survival.

Key Words
- triple-negative breast cancer
- Hippo signaling
- TAZ oncoprotein
- epithelial–mesenchymal transition

Endocrine-Related Cancer (2015) **22**, 443–454

Introduction

The Hippo pathway acts as a major regulator of tissue growth and organ size, and it has important implications in cancer (Pan 2010, Harvey *et al.* 2013). The final downstream transducers of the Hippo pathway are the transcriptional coactivators TAZ and YAP, both of which contribute to epithelial–mesenchymal transition (EMT)-mediated

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DOI: 10.1530/ERC-14-0456

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cancer progression. A bidirectional relationship between YAP/TAZ and EMT, wherein a loss of cell polarity and cell–cell junctions induces the activation of both factors that in turn sustain the EMT program, has been proposed (Piccolo & Cordenonsi 2013). The activity of TAZ/YAP is mainly inhibited by phosphorylation mediated by the LATS1/2 kinases, which in turn are activated by the MST1/2 kinases (homologues of *Drosophila* Hippo). Upon phosphorylation, TAZ/YAP undergo ubiquitination-mediated degradation and/or are sequestered in the cytoplasm by anchoring proteins (Pan 2010, Zhao *et al*. 2011). Elevated TAZ or YAP expression and nuclear localization have been observed in a broad range of different human cancers, and they often correlate with poor patient prognosis (Zender *et al*. 2006, Chan *et al*. 2008, Steinhardt *et al*. 2008, Bhat *et al*. 2011, de Cristofaro *et al*. 2011, Xie *et al*. 2012, Wang *et al*. 2013). In breast cancer, TAZ seems to be the relevant Hippo effector, because TAZ but not YAP protein expression appeared to be modulated in breast cancer cell lines (Chan *et al*. 2008). Indeed, TAZ is required for sustaining self-renewal and tumor-initiation capacities in breast cancer stem cells (Cordenonsi *et al*. 2011), and it is a central mediator of the metastatic ability of those stem cells (Bartucci *et al*. 2014). Increased protein expression of TAZ has been found in high-grade breast carcinomas, and it has been shown to have an influence on patient prognosis (Bartucci *et al*. 2014). However, the analysis of TAZ protein expression across different breast cancer phenotypes has not, to our knowledge, been inspected thus far. In order to better understand the role of TAZ in the development and progression of breast cancer, we analyzed TAZ immunohistochemical expression in a large retrospective cohort of 640 tumors, comprising 441 estrogen receptor (ER)-positive (ER+) tumors, 72 HER2-positive (HER2+)/ER-negative (ER−)/progesterone receptor (PR)-negative (PR−) tumors, and 127 triple-negative (TN) tumors. In addition, we evaluated the role of gene amplification and promoter DNA methylation in the regulation of TAZ expression in breast cancer.

Methods

Human tumors

A series of 640 formalin-fixed paraffin-embedded (FFPE) human invasive breast carcinomas, including some cases from a previous study (Castilla *et al*. 2014) and an independent tumor set of 15 metaplastic breast carcinomas (MBC), were obtained from the archives of the Department of Pathology of the Hospital Universitario Virgen del Rocío, Sevilla, Spain. The tumors had been diagnosed between 2007 and 2011, and the only selection criteria were the availability of pathological data and tissue for tissue microarray (TMA) construction. Medical records were retrospectively reviewed for clinicopathologic information (summarized in Table 1). Information regarding stage, grade, tumor size, and nodal status was abstracted. For the mRNA expression and DNA methylation studies, we used frozen sections of a cohort subset of 51 breast tumor specimens, in which the tumor component represented more than 70% of the cells. Both the FFPE and frozen samples were provided by the Biobanco del Sistema Sanitario Público de Andalucía.

Survival analysis was performed on a group of 483 invasive breast carcinoma patients for whom clinical data were available. In that group, 322 (66.6%) patients had ER+/PR+ carcinomas, 48 (9.9%) had HER2+ carcinomas, and 113 (23.4%) had TN carcinomas. After surgery, the patients were treated with chemotherapy, adjuvant radiotherapy, hormonal therapy, and/or trastuzumab according to their respective disease characteristics. Briefly, 295 of the 322 (91.6%) patients with ER+ carcinomas were treated with hormone therapy (tamoxifen or aromatase inhibitor). A total of 289 patients were treated with chemotherapy: 207 patients (71.6%) with anthracyclines plus taxanes, 75 (25.6%) with anthracyclines only, and seven (2.4%) with taxanes. An additional patient received chemotherapy that did not involve anthracycline or taxane administration. Of the patients with HER2+ tumors, 39 of 48 (81%) were treated with trastuzumab.

The present study was performed in accordance with the standard ethical procedures dictated by Spanish law (Ley de Investigación Orgánica Biomédica, 14 July 2007), and it was approved by the ethics committee of the Hospital Virgen del Rocío de Sevilla and the Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla (FISEVI), Spain. Written informed consent was obtained from all of the patients, and all of the clinical analyses were conducted in accordance with the principles of the Helsinki Declaration.

TMA construction and immunohistochemistry

Representative areas of the breast tumors were carefully selected on H&E-stained sections, and two 1 mm diameter tissue cores were obtained from each specimen. The cores were precisely arrayed into new paraffin blocks using a TMA workstation (Beecher Instruments, Sun Prairie, WI, USA). immunohistochemistry (IHC) was carried out on TMA sections using the Envision method (Dako, Glostrup,

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Table 1 Clinical and pathological findings according to TAZ expression ($n=640$)

Characteristics	Analyzable	TAZ expression (IHC)		P
		Negative ($n=496$)	Positive ($n=145$)	
Age (years)	639	58.71±13.6	58.40±13.1	0.914
Histologic type	634			0.403
Ductal carcinoma	573	444 (77.5%)	129 (22.5%)	
Lobular carcinoma	33	27 (81.8%)	6 (18.2%)	
Others	28	19 (67.9%)	9 (32.1%)	
pT (tumor size)	633			0.445
1	298	238 (79.9%)	60 (20.1%)	
2	281	210 (74.7%)	71 (25.3%)	
3	36	26 (72.2%)	10 (27.8%)	
4	18	14 (77.8%)	4 (22.2%)	
Nodal status	627			0.451
N0	327	249 (76.1%)	78 (23.9%)	
N1/N2/N3	300	236 (78.7%)	64 (21.3%)	
Pathological stage	639			0.426
I	218	172 (78.9%)	46 (21.1%)	
II	276	205 (74.3%)	71 (25.7%)	
III	141	114 (80.9%)	27 (19.1%)	
IV	4	3 (75.0%)	1 (25.0%)	
Histological grade (differentiation)	625			**<0.001**
1 and 2 (well/moderate)	297	258 (86.9%)	39 (13.1%)	
3 (poor)	328	225 (68.6%)	103 (31.4%)	
%Ki67	624			**<0.001**
<14	470	400 (85.1%)	70 (14.9%)	
≥14	154	83 (53.9%)	71 (46.1%)	
ER	640			**<0.001**
Negative	203	111 (54.7%)	92 (45.3%)	
Positive	437	384 (87.9%)	53 (12.1%)	
PR	635			**<0.001**
Negative	243	146 (60.1%)	97 (39.9%)	
Positive	392	344 (87.8%)	48 (12.2%)	
HER2	640			0.421
Negative	524	402 (76.7%)	122 (23.3%)	
Positive	116	93 (80.2%)	23 (19.8%)	
Receptor phenotype	640			**<0.001**
ER+/PR+	441	388 (88.0%)	53 (12.0%)	
HER2	72	58 (80.6%)	14 (19.4%)	
TN	127	49 (38.6%)	78 (61.4%)	
Molecular phenotype	640			**<0.001**
Luminal A	299	261 (87.3%)	38 (12.7%)	
Luminal B	98	92 (93.9%)	6 (6.1%)	
ER+/PR+ HER2+	44	35 (79.5%)	9 (20.5%)	
HER2	72	58 (80.6%)	14 (19.4%)	
TN-NOS	62	30 (48.4%)	32 (51.6%)	
Basal	65	19 (29.2%)	46 (70.8%)	

ER, estrogen receptor; PR, progesterone receptor; TN, triple-negative; TN-NOS, triple-negative not otherwise specified.

Denmark) with primary antibodies against TAZ, ER, PR, HER2, KI67, CK5/6, CK17, and EGFR. Myoepithelial staining of p63 was used to test the presence of *in situ* component in a subset of 11 TN tumors. For molecular classification, breast carcinomas were grouped according to immunohistochemical criteria as a surrogate definition of the intrinsic subtypes of breast cancer (Goldhirsch *et al.* 2011): tumors showing ER or PR expression and no HER2 expression were regarded as luminal A or B depending on their KI67 index (<14 or ≥14 respectively); tumors with ER or PR and HER2 expression were classified as ER+/PR+/HER2+; and TN tumors with CK5/6, CK17, or EGFR expression were ascribed to the basal phenotype, whereas all other TN breast carcinomas were termed TN-not otherwise specified (NOS).

Details of the clones, suppliers, dilutions, and scoring criteria used are provided in Supplementary Table 1, see section on supplementary data given at the end of

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this article. IHC staining was separately evaluated by two pathologists (J P and M A L). For TAZ, IHC evaluation was carried out for nuclear staining, thus focusing on the transcriptional activity of the factor. Tissue was given a score of 0 (negative) to 3 based on the percentage of positive cells (0: $<5\%$; 1: $\geq 5\%$ and $<30\%$; 2: $\geq 30\%$ and $<70\%$; 3: $\geq 70\%$). Images of the immunohistochemical markers used in Fig. 1 were captured using an Olympus BX61 microscope and DP72 camera, with CellSens CELL-A Software (Olympus, Hamburg, Germany).

Fluorescence *in situ* hybridization

We carried out fluorescence *in situ* hybridization (FISH) analysis on sequential 3 μm sections of TMAs containing 30 TN tumors. To detect *TAZ* amplification, we used the bacterial artificial chromosome RP11-126i10, chr3: 150 717 532 – 150 892 549 (Children's Hospital Oakland Research Institute, Oakland, CA, USA). A chromosome enumeration probe (CEP) 3 (Vysis, Abbott Laboratories) was included in dual hybridization with the TAZ probe.

Slides were deparaffinized, boiled in a pressure cooker with ethylene diaminetetraacetic acid (1 mM, pH 8.0) for 10 min, and incubated with pepsin at 37 °C for 10 min. The slides were then dehydrated. The probes were denatured at 75 °C for 2 min and simultaneously applied to the sample. After overnight hybridization at 37 °C in a humid chamber, the slides were washed with 0.4×SSC and 0.3% NP40 and counterstained with 0.2 μM 4′, 6′-diamino-2′-phenyl indole. Cell images were captured with a CCD camera (Photometrics SenSys, Tucson, AZ, USA) connected to a PC running Chromofluor image analysis software (Applied Imaging Ltd, Newcastle, UK). Samples showing sufficient FISH efficiency ($>90\%$ of nuclei with signals) were evaluated. Signals were scored for at least 50 non-overlapping, intact nuclei. Non-neoplastic cells present in the specimen were used as a control. Specimens were considered to be amplified when the gene–CEP ratio was >2.5 in at least 20% of the tumor cells.

Real-time quantitative RT-PCR

Total RNA was extracted from selected areas of frozen tissue containing $>70\%$ tumor cells using a mirVana miRNA isolation kit (Ambion, Austin, TX, USA) after the tissue had been homogenized for 2 min in lysis buffer using an Ultra-turrax (T10 Basic, IKA, Satufen, Germany) according to the manufacturer's protocol. The real-time quantitative RT-PCR (qRT-PCR) for *WWTR1* and *YAP1* was performed with gene-specific fluorescent Taqman probes (Taqman Gene Expression Assays, Applied Biosystems), using *MRPL19* as the endogenous control to normalize for variations in the quantity of the input cDNA. Details of qRT-PCR have been previously described (Castilla *et al.* 2012, 2014).

DNA methylation analysis by MassARRAY

DNA methylation analysis of *TAZ* and *YAP1* loci by MassARRAY (Sequenom, San Diego, CA, USA) was performed as previously described (Castilla *et al.* 2012). Three amplicons were designed for each promoter region, covering 1008 pb (*TAZ*) and 1465 pb (*YAP1*) respectively. Primer sequences, chromosomal locations, and number of CpG units analyzed are shown in Supplementary Table 2, see section on supplementary data given at the end of this article. The DNA methylation level of the promoter sequences was calculated by averaging the methylation levels of the individual CpG units.

Cell lines and western blot

Six breast cancer cell lines with different phenotypes – MCF7, T47-D, MDA-MB-231, BT-549, BT-474, and SK-BR-3 – were obtained from the American Tissue Culture

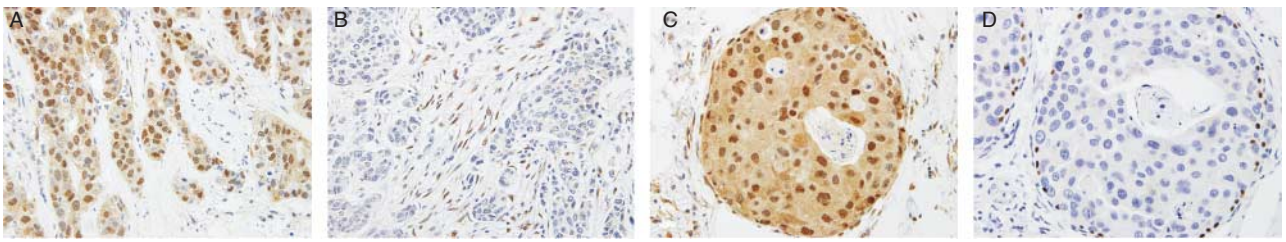

Figure 1
Immunohistochemical staining of TAZ protein in breast tumors. (A) Representative image for TAZ-positive expression in basal breast tumors. Intense nucleus staining and faint signal in cytoplasm is observed. (B) TAZ-negative expression in a luminal breast tumor. Staining of myofibroblastic cells can be observed within the tumor stroma. (C) TAZ staining in the *in situ* component of a TN breast carcinoma. (D) p63 expression in myoepithelial cells delimiting the *in situ* component; same sample as in (C).

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Collection and cultured in the recommended media. Whole cell extracts and western blotting procedure were performed as described previously (Moreno-Bueno *et al.* 2009). Signals were visualized using Immun-Star Western C kit (Bio-Rad). For TAZ detection, we used rabbit polyclonal anti-TAZ (Sigma, HPA007415, 1:250 diluted), and as loading control, we used GAPDH (Cell Signaling Technology (Danvers, MA, USA), (D16H11) XP Rabbit mAb, 1:2500 diluted).

The Cancer Genome Atlas dataset

mRNA expression *z*-scores (microarray data), methylation β-values, and putative copy-number alterations (GISTIC) for The Cancer Genome Atlas (TCGA 2012) dataset were obtained from cBioPortal for Cancer Genomics (http://www.cbioportal.org/). Tumors ($n=507$) were grouped into subtypes according to the PAM50 gene expression signature (Parker *et al.* 2009) (luminal A, $n=234$; luminal B, $n=134$; HER2, $n=58$; basal, $n=81$).

Statistical analysis

The correlation between inmunohistochemical TAZ expression and clinicopathological characteristics was assessed by χ^2 tests for the categorical variables (summarized with percentages). Mann–Whitney *U* tests were used for the analysis of differences with respect to the continuous variable age (summarized with means and S.D.). Breast-cancer-specific survival was defined as the time from surgery to the time of death from breast cancer, with deaths from other causes being censored, whereas in the time to relapse analysis, the endpoint was breast cancer recurrence, either local or distant. Survival curves were estimated using the Kaplan–Meier method, and the differences in survival were evaluated using the log-rank test. Cox's proportional hazards modeling of parameters potentially related to survival was conducted to calculate hazard ratios (HRs) in both univariate and multivariate analyses. All of these statistical analyses were performed using SPSS version 20 (SPSS Inc.) and JMP 10 statistical software (SAS Institute, Inc., Cary, NC, USA). $P<0.05$ was considered statistically significant.

The statistical significance of relative changes in mRNA expression between different groups of breast tumors was determined by the Wilcoxon rank-sum test, and *P* values were corrected with the Benjamini–Hochberg algorithm ($P<0.05$). A moderate *t*-test was used for analyzing statistical differences in the cell line expression data. These analyses were conducted using the Integromics Real Time StatMiner 4.5 package (Integromics S.L., Granada, Spain). Differences in DNA methylation levels among the distinct breast cancer phenotypes or cell lines were assessed by ANOVA.

Results

TAZ IHC expression is associated with the TN phenotype

We analyzed TAZ expression by IHC in a retrospective series with a total of 640 invasive human breast carcinomas (Table 1). TAZ-expressing tumor cells exhibited intense nuclear staining with a variable fainter signal in the cytoplasm (Fig. 1). TAZ expression was also observed in myofibroblastic cells in TAZ-negative tumor samples, which provided an internal positive control for the IHC determination (Fig. 1B). Table 1 shows the relationship between TAZ IHC expression and clinicopathological factors. TAZ-positive expression was associated with poorly differentiated tumors, high Ki67 index, and ER−/PR− phenotype, whereas no significant association was observed with age at surgery, histological type, tumor size (pT), nodal status, or pTNM stage. Grouping tumor specimens with reference to the receptor phenotype unveiled a clear association of TAZ IHC expression with the TN phenotype (Table 1). Grade distribution among TN and HER2 tumors is biased towards poor differentiation (Supplementary Table 3, see section on supplementary data given at the end of this article), but whereas 61.4% of TN tumors are TAZ-positive, only 19.4% of HER2 tumors showed TAZ expression (Table 1). Moreover, when only grade 3 tumors were compared, the strong association of TAZ IHC expression with the TN phenotype remained, with ER+/PR+ tumors exhibiting a low frequency of TAZ positivity (11.2%, Supplementary Table 4). Therefore, the relationship between TAZ expression and TN phenotype would not be ascribable to an underlying association with the differentiation grade. Interestingly, further stratification of tumor phenotype into a molecular classification revealed a striking association of TAZ expression to the basal phenotype (70.8% TAZ-positive, Table 1). Given the established link between TAZ/YAP and EMT (Piccolo & Cordenonsi 2013), we assessed TAZ expression in an independent tumor set of 15 MBC. Interestingly, 11 out of 12 MBC with histological evidence of EMT toward spindle cell or heterologous sarcomatous differentiation showed TAZ expression; in contrast, two out of three MBC with squamous differentiation were TAZ-negative (Table 2). However, the small sample size precludes detection of a statistically significant difference ($P=0.08$, Fisher's exact test).

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Table 2 TAZ nuclear expression in metaplastic breast carcinomas ($n=15$)

Metaplastic component	*n*	TAZ expression (IHC)	
		Negative	Positive
Chondrosarcoma	4	1 (25%)	3 (75%)
Osteosarcoma	1	0 (0%)	1 (100%)
Spindle cell	7	0 (0%)	7 (100%)
Squamous	3	2 (66%)	1 (33%)
Total	15	3 (20%)	12 (80%)

To investigate whether TAZ expression was restricted to the invasive tumor component, we also examined, by IHC, the expression of TAZ in the *in situ* component of selected TN tumors that had displayed either TAZ-positive (seven specimens) or TAZ-negative (four specimens) expression in the infiltrating component (Fig. 1C and D). We observed a full concordance of TAZ expression between the two components in all of the samples analyzed, with TAZ IHC signal detected in both the *in situ* and the infiltrating components of TAZ-positive specimens (Fig. 1C and D).

TAZ-encoding locus amplification in TN breast cancer

We next investigated whether amplification of *WWTR1* (the *TAZ*-encoding locus) could account for TAZ upregulation in TN/basal tumors. We performed FISH analyses of a subset of 30 TN tumors with strong TAZ IHC expression in which only two tumors (6.5%) exhibited amplification of the *WWTR1* locus (Fig. 2). We also interrogated the TCGA dataset for copy number variation in *WWTR1* (Supplementary Figure 1, see section on supplementary data given at the end of this article). These tumors are classified according to the PAM50 gene expression signature, and therefore, the different groups do not fully overlap those defined by IHC in the present series. Basal tumors in the TCGA dataset exhibited high-copy gains for the *WWTR1* locus with a higher proportion (7.4%) than luminal or HER2 tumors (0.5–0.8%). However, the available data on copy number estimates for *WWTR1* in breast cancer cell lines (Reinhold *et al.* 2012) revealed no evident amplification of the locus either in basal or luminal cell lines (data not shown).

TAZ mRNA expression is not modulated by DNA methylation in breast cancer

We also investigated whether the DNA methylation status of *WWTR1*, and the closely related transcription coactivator *YAP1*, is an operating epigenetic regulatory mechanism in breast cancer. First, we quantified mRNA expression levels of *TAZ* and *YAP1* in a series of 51 frozen samples from tumors included in our original cohort. This subset comprised 26 ER+/PR+ tumors and 25 TN tumors. We detected a significant twofold increase of *TAZ* mRNA in TN tumors ($P=0.0134$), and a slight but not significant increase was also observed for *YAP1* ($P=0.0774$) (Fig. 3A). Next, we inspected broad enriched CpG regions for DNA methylation of the *TAZ* and *YAP1* loci. Our analysis covered 65 CpG dinucleotides along 1008 bp of the *TAZ* promoter region and 113 CpG dinucleotides along 1465 bp of the *YAP1* promoter region. Minimal differences in the average percentage of CpG methylation levels were detected (TAZ, $P=0.0299$; YAP1, $P=0.0496$), with 11 and 13% in the ER+/PR+ and TN groups respectively (Fig. 3B).

We also analyzed *TAZ* and *YAP1* mRNA expression and DNA methylation levels in the breast cancer dataset from the TCGA (2012). In accordance with the observations in our series, we detected upregulation of both *TAZ* and *YAP1* mRNA in basal tumors, with *TAZ* showing a higher increase (Supplementary Figure 2). Moreover, only minimal differences were observed in the DNA methylation levels of the two loci between different tumor subgroups (Supplementary Figure 2). In fact, no correlation between expression and DNA methylation data was observed in the TCGA dataset or in our tumor series (data not shown).

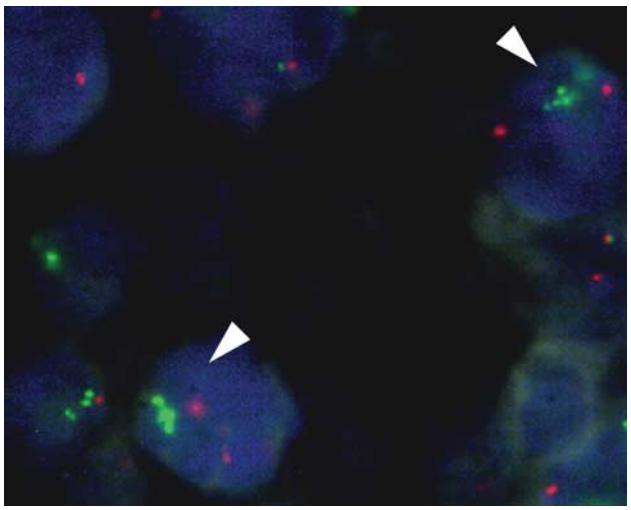

Figure 2
Positive FISH amplification for the *WWTR1* locus. DAPI staining for the nucleus (blue), Spectrum Green Probe for *WWTR1*, and Spectrum Orange Probe for the centromere of chromosome 3.

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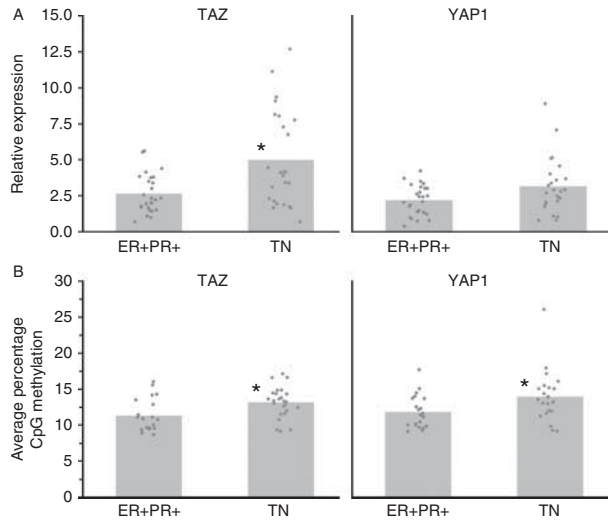

Figure 3
TAZ and *YAP1* mRNA expression (A) and DNA methylation levels (B) in a subset of 51 breast tumors (freeze samples). Points represent relative values for individual cases. Bars represent the mean for each group (ER+/PR+ tumors, $n=26$; TN tumors, $n=25$; $^*P<0.05$).

To further confirm these observations, we analyzed a panel of breast cancer cell lines representing the luminal (MCF7, T47D), HER2 (SK-BR-3, BT-474), and basal phenotypes (MDA-MB-231, BT-549). As previously reported by Chan *et al*. (2008), we observed higher expression levels of TAZ protein in basal cell lines (Fig. 4A). Moreover, expression levels of *TAZ* mRNA as well as *YAP1* mRNA were higher in basal cell lines (e.g., BT-549 versus T-47D, $P<0.0001$ and $P=0.002625$ for *TAZ* and *YAP1* mRNA expression respectively), although *YAP1* mRNA modulation across the cell line panel was not as evident as *TAZ* mRNA variation was (Fig. 4B). Finally, similar DNA methylation levels for both *TAZ* (below 5%, $F=0.432$, $P=0.826$) and *YAP1* (below 7%, $F=0.508$, $P=0.770$) were observed for the cell lines of different phenotypes (Fig. 4C).

TAZ IHC expression affects survival

Given the association observed between TAZ and the TN/basal phenotype, and because TN/basal phenotypes have a poorer prognosis as compared with luminal phenotypes, we tested the prognostic significance of TAZ expression in our cohort. We retrieved follow-up data for 483 out of 640 patients (median duration of follow-up, 53.7 months). Kaplan–Meier estimates of breast-cancer-specific survival were significantly shorter for the TAZ-positive group as compared with the TAZ-negative group (mean, 69.6 versus 79.6 months, $P<0.001$, Fig. 5A). Because a similar trend was observed for TAZ IHC scores 0 and 1 versus scores 2 and 3 at the initial follow-up

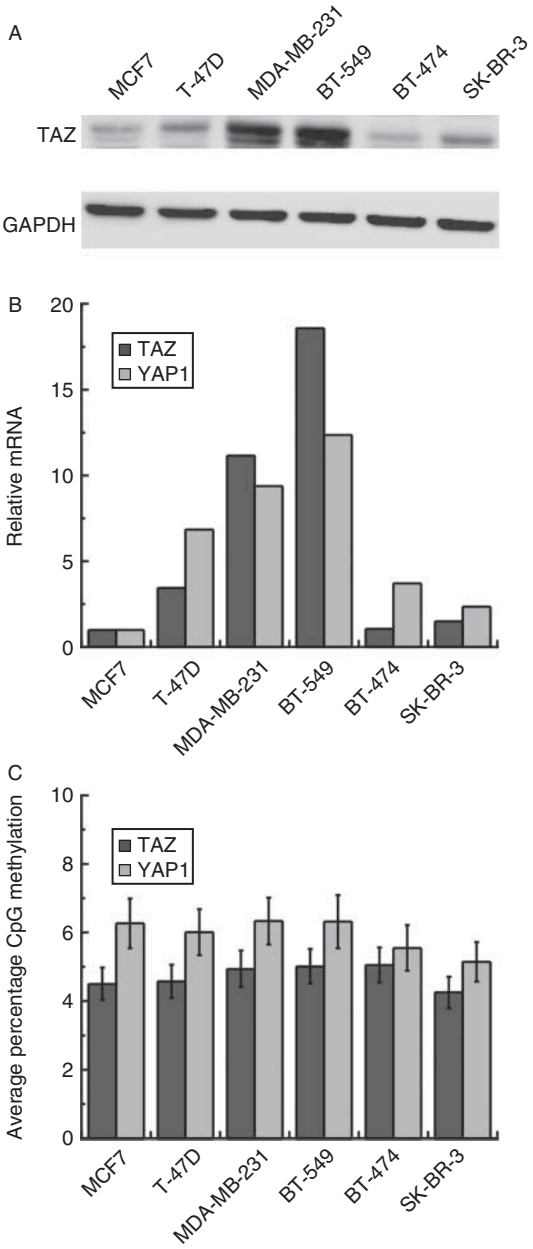

Figure 4
TAZ overexpression in basal cell lines is not correlated with DNA methylation levels. (A) Lysates from a panel of six breast cancer cell lines were probed for TAZ. The levels of GAPDH were used as internal loading controls. (B) Relative *TAZ* and *YAP1* mRNA expression levels determined by qRT-PCR. Data are normalized to the expression of *MRPL19*. Bars represent mean expression changes (triplicate measurements) relative to MCF7 cells (baseline). (C) DNA methylation status of *TAZ* and *YAP1* loci in the cell line panel as determined by MassArray. Histogram bars represent averaged percentage CpG methylation levels (±S.E.M.) in two replicates for each cell line.

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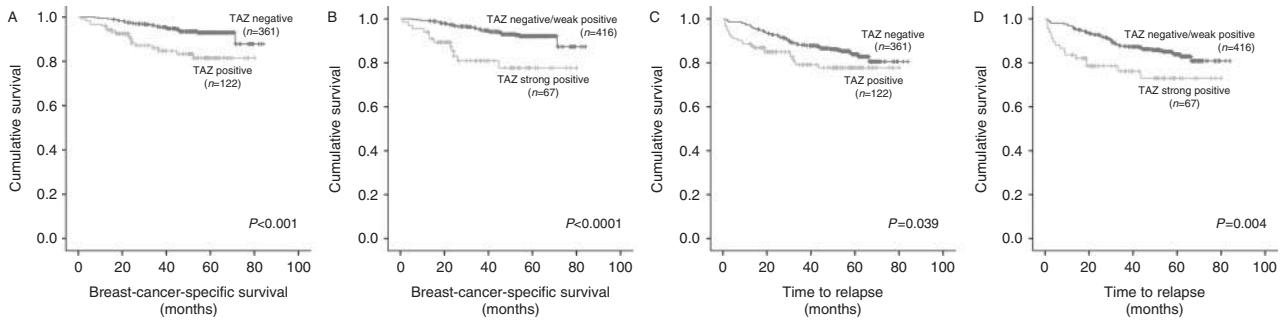

Figure 5
Kaplan–Meier survival curves for TAZ protein expression in breast cancer patients. (A and B) Breast-cancer-specific survival analyses with different patient grouping criteria regarding TAZ IHC intensity scoring. (C and D) Time to relapse analyses with the same grouping criteria.

period (Supplementary Figure 3, see section on supplementary data given at the end of this article), we grouped patients into those with strong TAZ expression (scores 2 and 3) and TAZ-negative or weak TAZ expression (scores 0 and 1). This stratification resulted in greater differences in survival estimates between the two patient groups (mean, 66.6 versus 79.2 months, $P<0.0001$, Fig. 5B). Similarly, TAZ expression also influenced significantly the time to relapse, which was shorter in TAZ-positive patients than it was in TAZ-negative patients (mean, 65.6 versus 74.2 months, $P=0.039$, Fig. 5C). Again, greater differences were observed when comparing the strong TAZ expression group with the TAZ-negative or weak TAZ expression group (mean, 62.1 versus 74.1 months, $P=0.004$, Fig. 5D).

Accordingly, using Cox regression univariate analyses (Table 3) it was determined that strong TAZ expression was significantly correlated with breast cancer–specific survival and the time to relapse, with the unadjusted HRs being 3.739 ($P<0.001$) and 2.244 ($P=0.004$) respectively. A significant correlation with survival and time to relapse was also observed for the classical prognostic factors analyzed (Table 3). These significant variables were all included simultaneously in order to assess the independent prognostic significance based on multivariate analysis (Table 3). The adjusted HR of strong TAZ expression for breast cancer–specific survival was 2.191 ($P=0.044$) after controlling the Cox regression model for the effects of stage, grade, ER, PR, tumor size, nodal status, and Ki index (Table 3). This indicates that TAZ expression is an independent and significant predictor of poorer survival. We could not definitely conclude the same for time to relapse, even though the level of significance nearly reached 0.05 (Table 3).

Discussion

The results of the present study clearly establish that TAZ oncoprotein expression is associated with the TN phenotype of breast cancer, and this correlation is not ascribable to the differentiation grade. Hence, the association of TAZ IHC expression with high-grade breast cancer that has been reported in previous studies (Cordenonsi *et al*. 2011, Bartucci *et al*. 2014) reflects instead the actual correlation with the receptor status. Skibinski *et al*. (2014) identified TAZ in a screen of transcription factors that regulate the differentiation state of mammary epithelial cells. Overexpression of TAZ was sufficient to induce the transition of luminal cells to a basal cell fate, and conversely, TAZ depletion in basal cells led to luminal differentiation (Skibinski *et al*. 2014).

Furthermore, TAZ IHC staining was observed both in the *in situ* and in the infiltrating components of TAZ-positive tumor specimens. Most *in situ* breast carcinomas (without an invasive component) exhibit an ER+/PR+ or an HER2+ phenotype, and therefore low TAZ positivity is observed among them. This may have led to the notion that TAZ expression is only associated with invasive ductal carcinomas (Chan *et al*. 2008). The stratification of TN tumors into basal and non-basal (TN-NOS) groups brought out preferential TAZ positivity in basal tumors (70.8%, $P<0.001$). In our series, 50% (65/129) of the TN tumors were identified as basal tumors using additional IHC markers. There is a substantial overlap between TN and basal-like definitions, and thus TN has often been assimilated as a surrogate for basal-like breast cancer (Foulkes *et al*. 2010, Badve *et al*. 2011). However, not all TN tumors would be identified as basal-like by their gene expression, and not all basal-like tumors are TN. It has

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Table 3 Prognostic value of TAZ IHC expression in relation to traditional prognostic variables in breast cancer ($n=483$). Adjusted HR refers to the multivariate model after controlling for the significant factors in the univariate analysis

	Breast-cancer-specific survival				Time to relapse			
Factors	Unadjusted HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*	Unadjusted HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
TAZ (strong versus negative/weak)	3.739 (1.898–7.366)	<**0.001**	2.191 (1.021–4.707)	**0.044**	2.244 (1.291–3.900)	**0.004**	1.755 (0.931–3.306)	0.082
Age	0.994 (0.971–1.018)	0.633			0.985 (0.967–1.002)	0.09		
ER (negative versus positive)	3.986 (2.116–7.506)	<**0.001**	3.087 (0.483–19.744)	0.234	2.335 (1.489–3.660)	<**0.001**	1.189 (0.436–3.243)	0.735
PR (negative versus positive)	3.444 (1.816–6.530)	<**0.001**	0.364 (0.053–2.514)	0.305	2.238 (1.428–3.508)	<**0.001**	0.939 (0.336–2.262)	0.905
HER2 (positive versus negative)	1.207 (0.552–2.640)	0.637			1.424 (0.821–2.470)	0.209		
Tumor size (pT1 versus pT2+pT3+pT4)	8.699 (3.098–24.430)	<**0.001**	4.132 (1.240–13.775)	**0.021**	3.963 (2.249–6.981)	<**0.001**	2.240 (1.159–4.327)	**0.016**
Node status (pN0 versus pN1,2,3)	5.062 (2.338–10.960)	<**0.001**	2.179 (0.874–5.430)	0.095	4.163 (2.452–7.067)	<**0.001**	1.870 (0.988–3.539)	0.055
Clinical stage (I+II versus III+IV)	6.605 (3.525–12.378)	<**0.001**	3.435 (1.643–7.184)	**0.001**	5.022 (3.210–7.859)	<**0.001**	2.740 (1.574–4.771)	<**0.001**
Grade (poorly versus well/moderately differentiated)	12.615 (3.890–40.912)	<**0.001**	6.266 (1.414–27.771)	**0.016**	4.630 (2.590–8.277)	<**0.001**	2.803 (1.408–5.586)	**0.003**
Ki67 (<14 versus ≥14)	6.025 (3.150–11.523)	<**0.001**	2.447 (1.153–5.194)	**0.020**	2.724 (1.717–4.321)	<**0.001**	1.320 (0.759–2.296)	0.326

ER, estrogen receptor; PR, progesterone receptor.

been shown that 71–78% of TN cancers exhibit a basal gene expression signature (Bertucci *et al.* 2008, Prat *et al.* 2013). Hence, our TN group of tumors might include unidentified basal specimens that may exhibit TAZ expression, and thus the ratio of TAZ-positive specimens in our basal group could be even higher than was observed (Table 1). This striking association of TAZ protein expression with the basal phenotype is a relevant finding, because it may contribute to the immunohistochemical/molecular characterization of these tumors. Patients with TN/basal breast cancer have a poor outcome because of the intrinsic aggressiveness of the cancer and the lack of effective targeted therapies (Duffy *et al.* 2012, Bayraktar & Gluck 2013). Therefore, the identification of specific biomarkers/molecular targets is imperative. In this regard, we have recently reported that *Vgll1*, a transcriptional cofactor with structural similarities to *TAZ* and *YAP1*, is mainly expressed in a subset of sporadic and BRCA1-associated TN/basal breast carcinomas (Castilla *et al.* 2014). In a recent report by Bartucci *et al.* (2014), TAZ IHC expression in a cohort of 99 tumors was not significantly correlated with receptor status, although the authors did observe an association between high tumor grade and Ki67 index, as we have observed in the present study. The limited size of the series or the different antibodies used could account for this discrepancy.

The mechanisms responsible for TAZ overexpression in human tumors are poorly understood, although both gene amplification and promoter methylation have been reported in some subsets of tumors (Bhat *et al.* 2011, Cordenonsi *et al.* 2011). The present study is the first to use FISH to analyze the role of gene amplification, and our results indicated, as previously reported by Cordenonsi *et al.* (2011), that amplification of the *TAZ*-encoding locus occurred only in a small proportion of breast carcinomas. Also in agreement with the present results, an analysis of the TCGA dataset indicated that high-level amplification of the *WWTR1* locus occurs in only 2% of breast carcinomas, although within the basal subgroup, the frequency increases to 7.4% (Supplementary Figure 1), a frequency similar to that observed in the present study but in contrast with the 60–70% of TAZ-positive TN/basal tumors observed by IHC. In fact, upregulation of TAZ mRNA levels were observed in 34 out of the 81 TN tumors (44%) in the TGCA dataset, which greatly exceeds the proportion of TAZ-amplified cases (Supplementary Figure 1).

To date, few somatic or germline mutations have been discovered in Hippo pathway genes (Harvey *et al.* 2013), which indicates that the frequent perturbation of the activity of the pathway in human cancer results from other mechanisms. In breast cancer, only three *TAZ* mutations in five unique samples are registered in the Catalogue of Somatic Mutations in Cancer, which represents only 0.51% of the total unique samples (Ding *et al.* 2010, Shah *et al.* 2012). Epigenetic regulation of *TAZ* by DNA methylation also did not explain the differential expression of *TAZ* among different phenotypes of breast cancer (Fig. 3, Supplementary Figure 2, see section on

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supplementary data given at the end of this article). This contrasts with results described in a recent report on glioblastoma, which indicated that *TAZ* expression correlated with the DNA methylation status of the promoter sequences (Bhat *et al*. 2011). This difference in *TAZ* deregulation might indicate that the mechanisms that modulate Hippo signaling are tissue- or cell-type-specific. It has been proposed that enrichment of *YAP/TAZ* target gene expression in high-grade breast cancer may be mainly attributed to TAZ protein stabilization (Cordenonsi *et al*. 2011). Delocalization from the cell membrane of Scribble, an upstream component of the Hippo pathway, relieves LATS-dependent TAZ inactivation and consequently induces TAZ stabilization and nuclear translocation. Recently, integrin-linked kinase (ILK) has been shown to be involved in the suppression of the Hippo pathway. Knockdown of ILK in breast, prostate, and colon cancer cells results in the activation of the core kinases MST and LAST and the cytoplasm sequestration of TAZ and YAP1 (Serrano *et al*. 2013). In light of these results, it seems that enhanced TAZ protein expression in TN/basal breast cancer may result from post-transcriptional/translational regulatory mechanisms, although transcriptional regulation also clearly contributes to this process (Fig. 3A, Supplementary Figure 2, see section on supplementary data given at the end of this article).

In the present study, we showed that TAZ protein expression is significantly associated with a decreased survival rate and time to relapse. Results of multivariate analysis indicated that strong TAZ IHC expression had prognostic value for breast-cancer–specific survival, independent from classical factors such as tumor size, nodal status, histological grade, and pathological stage. Accordingly, Bartucci *et al*. (2014) similarly reported that TAZ expression had an effect on disease-free survival. Thus, TAZ may represent a potential selective therapeutic target for TN/basal breast cancer, which has a poor outcome because of the intrinsic aggressiveness of the disease and the lack of effective targeted therapies (Toft & Cryns 2011, Duffy *et al*. 2012, Bayraktar & Gluck 2013). The association between TAZ overexpression and poor prognosis in breast cancer could be related to the intrinsic properties acquired by neoplastic cells when the Hippo pathway is inactivated. Indeed, a direct link between stemness, EMT, cell polarity, and Hippo signaling has been demonstrated in breast cancer. Cordenonsi *et al*. (2011) demonstrated that EMT induces TAZ protein stabilization, which is required to sustain self-renewal and tumor-initiation capacities in breast cancer stem cells. Furthermore, the different signaling pathways that are involved in EMT, such as the transforming growth factor-β pathway and the Wnt pathway, are highly interconnected with the Hippo pathway, and there is a substantial degree of crosstalk between them (Varelas & Wrana 2012, Hiemer *et al*. 2014). In addition, it has recently been shown that TAZ endows metastatic abilities to breast cancer stem cells (Bartucci *et al*. 2014). We previously reported that basal tumors undergo EMT-like processes (Sarrio *et al*. 2008) that may be related to the high aggressiveness and the characteristic metastatic spreading of these tumors. Thus, we speculate that upregulation of TAZ protein in basal tumors could be regarded as part of the EMT program that occurs in this tumor subtype. Indeed, we have shown that MBC with sarcomatous differentiation exhibited TAZ protein expression (Table 2). In this context, Lehmann *et al*. (2011) reported that TAZ is upregulated in the mesenchymal-like subtype and downregulated in the luminal AR subtype (as compared with the rest of the subtypes; Supplementary Table 3 in Lehmann *et al*. 2011). In the present paper, the mesenchymal-like subtype shared similar features with MBC. Therefore, our results for MBC (Table 2) were in accordance with this observation.

In summary, in this large series of breast carcinomas, we found a striking association of TAZ expression, determined by IHC, with the TN and basal phenotypes of breast cancer that was not ascribable to the underlying grade distribution in these tumor subtypes. Moreover, *TAZ* overexpression was even more frequent in those tumors with morphological EMT features, such as metaplastic carcinomas with spindle cell or hetrologous sarcomatous differentiation. *TAZ*-enhanced expression in TN/basal tumors was not correlated with DNA methylation, but amplification could contribute to *TAZ* upregulation in a small number of cases. Using Cox regression analyses, which included the classical prognostic parameters, it was determined that TAZ IHC expression could be a relevant prognostic biomarker in breast cancer.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/ERC-14-0456.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by grants to J Palacios from the Instituto de Salud Carlos III (ISCIII, grant number PI080971), the Ministerio de Ciencia e

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Innovación (MICINN), the European Development Regional Fund (grant number RD06/0020/0013), the Junta de Andalucía (Consejería de Salud, grant numbers PI-0384/2007 and PI0581/2009); and the ISCIII-Ministerio de Economía y Competitividad (MINECO, grant numbers RD12/0036/0064 and PI13/02477). J Díaz-Martín and M A Castilla are PhD researchers funded by the ISCIII (Sara Borrell and RD06/0020/0013 contracts respectively). L Romero-Pérez is a PhD student recipient of a Predoctoral de Formación en Investigación en Salud (PFIS) fellowship (grant number F109/ 00193). M L Pecero works as a laboratory technician supported by the ISCIII (PI080971). M R Atienza-Amores is an MD funded by the ISCIII (Río Ortega program).

Author contribution statement

J Díaz-Martín was responsible for the experimental design, the performance of the experiments, and the statistical analysis. M Biscuola and A Santón performed the statistical analyses. L Romero-Pérez, M L Pecero, and M A Castilla carried out some of the experiments. J Palacios and M A López-García reviewed the pathological and immunohistochemical analyses. M R Atienza-Amores reviewed the clinical data. J Díaz-Martín and J Palacios designed the study and drafted the manuscript. All of the authors contributed to the editing of the manuscript and gave their approval of the final version.

Acknowledgements

The authors thank the donors and the Andalusian Public Health System Biobank (ISCIII-Red de Biobancos RD09/0076/00085) for the human specimens used in this study.

References

Badve S, Dabbs DJ, Schnitt SJ, Baehner FL, Decker T, Eusebi V, Fox SB, Ichihara S, Jacquemier J, Lakhani SR *et al.* 2011 Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. *Modern Pathology* **24** 157–167. (doi:10.1038/modpathol.2010.200)

Bartucci M, Dattilo R, Moriconi C, Pagliuca A, Mottolese M, Federici G, Benedetto AD, Todaro M, Stassi G, Sperati F *et al.* 2014 TAZ is required for metastatic activity and chemoresistance of breast cancer stem cells. *Oncogene* **34** 681–690. (doi:10.1038/onc.2014.5)

Bayraktar S & Gluck S 2013 Molecularly targeted therapies for metastatic triple-negative breast cancer. *Breast Cancer Research and Treatment* **138** 21–35. (doi:10.1007/s10549-013-2421-5)

Bertucci F, Finetti P, Cervera N, Esterni B, Hermitte F, Viens P & Birnbaum D 2008 How basal are triple-negative breast cancers? *International Journal of Cancer* **123** 236–240. (doi:10.1002/ijc.23518)

Bhat KP, Salazar KL, Balasubramaniyan V, Wani K, Heathcock L, Hollingsworth F, James JD, Gumin J, Diefes KL, Kim SH *et al.* 2011 The transcriptional coactivator TAZ regulates mesenchymal differentiation in malignant glioma. *Genes and Development* **25** 2594–2609. (doi:10.1101/gad.176800.111)

Castilla MA, Diaz-Martin J, Sarrio D, Romero-Perez L, Lopez-Garcia MA, Vieites B, Biscuola M, Ramiro-Fuentes S, Isacke CM & Palacios J 2012 MicroRNA-200 family modulation in distinct breast cancer phenotypes. *PLoS ONE* **7** e47709. (doi:10.1371/journal.pone.0047709)

Castilla MA, Lopez-Garcia MA, Atienza MR, Rosa-Rosa JM, Diaz-Martin J, Pecero ML, Vieites B, Romero-Perez L, Benitez J, Calcabrini A *et al.* 2014 *VGLL1* expression is associated with a triple-negative basal-like phenotype in breast cancer. *Endocrine-Related Cancer* **21** 587–599. (doi:10.1530/ERC-13-0485)

Chan SW, Lim CJ, Guo K, Ng CP, Lee I, Hunziker W, Zeng Q & Hong W 2008 A role for TAZ in migration, invasion, and tumorigenesis of breast cancer cells. *Cancer Research* **68** 2592–2598. (doi:10.1158/0008-5472.CAN-07-2696)

Cordenonsi M, Zanconato F, Azzolin L, Forcato M, Rosato A, Frasson C, Inui M, Montagner M, Parenti AR, Poletti A *et al.* 2011 The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. *Cell* **147** 759–772. (doi:10.1016/j.cell.2011.09.048)

de Cristofaro T, Di Palma T, Ferraro A, Corrado A, Lucci V, Franco R, Fusco A & Zannini M 2011 TAZ/WWTR1 is overexpressed in papillary thyroid carcinoma. *European Journal of Cancer* **47** 926–933. (doi:10.1016/j.ejca.2010.11.008)

Ding L, Ellis MJ, Li S, Larson DE, Chen K, Wallis JW, Harris CC, McLellan MD, Fulton RS, Fulton LL *et al.* 2010 Genome remodelling in a basal-like breast cancer metastasis and xenograft. *Nature* **464** 999–1005. (doi:10.1038/nature08989)

Duffy MJ, McGowan PM & Crown J 2012 Targeted therapy for triple-negative breast cancer: where are we? *International Journal of Cancer* **131** 2471–2477. (doi:10.1002/ijc.27632)

Foulkes WD, Smith IE & Reis-Filho JS 2010 Triple-negative breast cancer. *New England Journal of Medicine* **363** 1938–1948. (doi:10.1056/NEJMra1001389)

Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B & Senn HJ 2011 Strategies for subtypes – dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. *Annals of Oncology* **22** 1736–1747. (doi:10.1093/annonc/mdr304)

Harvey KF, Zhang X & Thomas DM 2013 The Hippo pathway and human cancer. *Nature Reviews. Cancer* **13** 246–257. (doi:10.1038/nrc3458)

Hiemer SE, Szymaniak AD & Varelas X 2014 The transcriptional regulators TAZ and YAP direct transforming growth factor β-induced tumorigenic phenotypes in breast cancer cells. *Journal of Biological Chemistry* **289** 13461–13474. (doi:10.1074/jbc.M113.529115)

Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y & Pietenpol JA 2011 Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *Journal of Clinical Investigation* **121** 2750–2767. (doi:10.1172/JCI45014)

Moreno-Bueno G, Peinado H, Molina P, Olmeda D, Cubillo E, Santos V, Palacios J, Portillo F & Cano A 2009 The morphological and molecular features of the epithelial-to-mesenchymal transition. *Nature Protocols* **4** 1591–1613. (doi:10.1038/nprot.2009.152)

Pan D 2010 The hippo signaling pathway in development and cancer. *Developmental Cell* **19** 491–505. (doi:10.1016/j.devcel.2010.09.011)

Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, Davies S, Fauron C, He X, Hu Z *et al.* 2009 Supervised risk predictor of breast cancer based on intrinsic subtypes. *Journal of Clinical Oncology* **27** 1160–1167. (doi:10.1200/JCO.2008.18.1370)

Piccolo S & Cordenonsi M 2013 Regulation of YAP and TAZ by epithelial plasticity. In *The Hippo Signaling Pathway and Cancer*, pp 89–113. Eds M Oren & Y Aylon. New York, NY: Springer. (doi:10.1007/978-1-4614-6220-0_6)

Prat A, Adamo B, Cheang MC, Anders CK, Carey LA & Perou CM 2013 Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. *Oncologist* **18** 123–133. (doi:10.1634/theoncologist.2012-0397)

Reinhold WC, Sunshine M, Liu H, Varma S, Kohn KW, Morris J, Doroshow J & Pommier Y 2012 CellMiner: a web-based suite of genomic and pharmacologic tools to explore transcript and drug patterns in the NCI-60 cell line set. *Cancer Research* **72** 3499–3511. (doi:10.1158/0008-5472.CAN-12-1370)

Sarrio D, Rodriguez-Pinilla SM, Hardisson D, Cano A, Moreno-Bueno G & Palacios J 2008 Epithelial–mesenchymal transition in breast cancer relates to the basal-like phenotype. *Cancer Research* **68** 989–997. (doi:10.1158/0008-5472.CAN-07-2017)

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Serrano I, McDonald PC, Lock F, Muller WJ & Dedhar S 2013 Inactivation of the Hippo tumour suppressor pathway by integrin-linked kinase. *Nature Communications* **4** 2976. (doi:10.1038/ncomms3976)

Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, Turashvili G, Ding J, Tse K, Haffari G *et al*. 2012 The clonal and mutational evolution spectrum of primary triple-negative breast cancers. *Nature* **486** 395–399. (doi:10.1038/nature10933)

Skibinski A, Breindel JL, Prat A, Galvan P, Smith E, Rolfs A, Gupta PB, Labaer J & Kuperwasser C 2014 The Hippo transducer TAZ interacts with the SWI/SNF complex to regulate breast epithelial lineage commitment. *Cell Reports* **6** 1059–1072. (doi:10.1016/j.celrep.2014.02.038)

Steinhardt AA, Gayyed MF, Klein AP, Dong J, Maitra A, Pan D, Montgomery EA & Anders RA 2008 Expression of yes-associated protein in common solid tumors. *Human Pathology* **39** 1582–1589. (doi:10.1016/j.humpath.2008.04.012)

TCGA 2012 Comprehensive molecular portraits of human breast tumours. *Nature* **490** 61–70. (doi:10.1038/nature11412)

Toft DJ & Cryns VL 2011 Minireview: Basal-like breast cancer: from molecular profiles to targeted therapies. *Molecular Endocrinology* **25** 199–211. (doi:10.1210/me.2010-0164)

Varelas X & Wrana JL 2012 Coordinating developmental signaling: novel roles for the Hippo pathway. *Trends in Cell Biology* **22** 88–96. (doi:10.1016/j.tcb.2011.10.002)

Wang L, Shi S, Guo Z, Zhang X, Han S, Yang A, Wen W & Zhu Q 2013 Overexpression of YAP and TAZ is an independent predictor of prognosis in colorectal cancer and related to the proliferation and metastasis of colon cancer cells. *PLoS ONE* **8** e65539. (doi:10.1371/journal.pone.0065539)

Xie M, Zhang L, He CS, Hou JH, Lin SX, Hu ZH, Xu F & Zhao HY 2012 Prognostic significance of TAZ expression in resected non-small cell lung cancer. *Journal of Thoracic Oncology* **7** 799–807. (doi:10.1097/JTO.0b013e318248240b)

Zender L, Spector MS, Xue W, Flemming P, Cordon-Cardo C, Silke J, Fan ST, Luk JM, Wigler M, Hannon GJ *et al*. 2006 Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. *Cell* **125** 1253–1267. (doi:10.1016/j.cell.2006.05.030)

Zhao B, Tumaneng K & Guan KL 2011 The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal. *Nature Cell Biology* **13** 877–883. (doi:10.1038/ncb2303)

Received in final form 30 March 2015
Accepted 1 April 2015
Made available online as an Accepted Preprint
13 April 2015

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