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Aberrant methylation of yes-associated protein (*YAP*1) as a potential biomarker in breast cancer

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Abstract

Background: Breast cancer (BC) represents the most prevalent malignancy among women, and it is characterized by high mortality especially in late stages. BC tumorigenesis was linked to epigenetic alterations namely methylation. Yes-associated protein (YAP1) is the leading downstream effector of the Hippo pathway. It may enhance or inhibit oncogenesis based on the tissue involved.

Aim: This case-control study aimed to analyze the methylation degree of promoter region of *YAP*1 gene in BC patients by applying methylation-specific polymerase chain reaction (MSP) analysis.

Methods: Genomic deoxyribonucleic acid (DNA) was isolated from 50 paired tumor and adjacent noncancerous breast tissue samples and subjected to bisulfite conversion. Methylation condition of *YAP*1 gene was studied by MSP and evaluated as a possible biomarker for diagnosing BC and its differentiation from corresponding normal tissues. We also correlated the aberrant methylation with clinicopathological criteria.

Results: Increased methylation of the *YAP*1 gene promoter region in BC tumor tissue was detected in 68% of the studied BC tissue samples. There was a significant change in the frequency of *YAP*1 methylated genotype between breast tumor tissues compared to that in adjacent non-cancerous tissue ($p < 0.001$). *YAP*1 can discriminate early from late-stage BC with a sensitivity of 96.88% and specificity of 83.33%.

Conclusions: Gene analysis of *YAP*1 using conventional MSP in tissue specimens can be considered a possible biomarker to distinguish BC from normal breast tissue as well as between early- and late-stage BC.

Keywords: Breast cancer, Hippo pathway, Methylation-specific polymerase chain reaction analysis, Yes-associated protein (YAP1)

Background

Breast cancer (BC) is considered the most common malignancy in females, and it accounts for approximately 32.04% of the reported cancers in Egyptian women [1]. Cancer biomarkers' research has shown that not only genetic mutations, but also epigenetic alterations such as promoter methylation patterns have potential value in early recognition of malignant growth. Silencing of tumor suppressor genes through hypermethylation and activation of oncogenes by hypomethylation are hallmarks of early molecular epimarkers in BC development [2].

Mounting evidence emphasizes the significance of DNA methylation, not only for being potentially modifiable but also because it is related to age, the strongest BC risk predictor [3]. The Cancer Genome Atlas studied the whole genome methylation of more than 10,000 human samples from more than 30 distinct tumors and paired normal controls [4]. With the accessibility of such immense amount of genetic data, the challenge is to capture the most significant genes with relevance to diagnosis and personalized medicine.

One of the most frequent epigenetic alterations described is increased methylation of breast cancer gene (BRCA1). By using peripheral blood, BRAC1 promoter

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methylation could serve as a screening tool as well as predictive biomarker for poly ADP ribose polymerase (PARP1) inhibitor therapy, which has been used for management of BRCA1 and BRCA2 mutations to reduce tumor growth and vascularization [5, 6].

Yes-associated protein (*YAP*) gene is mapped to chromosome region 11q22 amplicon [7]. YAP1 is considered a specific transcriptional activator and a leading effector of the Hippo tumor suppressor pathway. The Hippo pathway is an evolutionarily conserved kinase cascade which is implicated in cell proliferation, organ development, regeneration, and stem cell biology. YAP1 regulates nuclear cellular responses by interacting with transcription factors. It also modulates actin filament architecture and cell migration [8, 9].

Hippo pathway is inhibited by distinct signaling pathways, and this causes hyperactivation of YAP1 with its subsequent shift to the nucleus. The hyperactivated YAP1 interacts with transcription regulators such as TEA domain/Transcription Enhancer Factor (TEAD) family. The complex between YAP1 and TEAD molecules is crucial for growth-enhancing function of YAP1 [10]. The mechanism by which YAP1 performs its tumor suppressor or oncogenic functions is not yet completely understood.

Aim

Regarding the aforementioned points, study of DNA methylation seems to be important in developing new diagnostic biomarkers; therefore, this work aimed at analyzing the methylation profile of *YAP*1 in BC patients using MSP analysis. Furthermore, the correlation of clinicopathological parameters and aberrant methylation was examined.

Methods

Fifty females, genetically unrelated, with primary BC undergoing breast surgery at the Medical Research Institute Teaching Hospital, Alexandria, Egypt, during the period 2016–2017, were included in our study. BC was diagnosed according to standard clinical, radiological, and histological parameters. Patients were subjected to thorough physical examinations, chest radiography, bilateral mammography, and ultrasonography of the breasts, axilla, cervical region, and abdomen before surgical resection. Breast tumor tissue and normal adjacent breast tissue not infiltrated with tumor, serving as control, were obtained from subjects during surgery. Exclusion criteria were previous exposure to neoadjuvant chemotherapy, radiotherapy, or hormonal therapy. Patients with multiple cancers and patients with acute myocardial or surgical complications were excluded. The tumor histological types, grading, and TNM staging were assessed [11]. Late stage was defined as stage III and stage IV. The study was approved by the Ethical Committee of Medical Research Institute, Alexandria University (IORG#: IORG008812). All subjects have signed the informed consent and agreed the research purposes of clinical data and samples of our study. Postoperative pathology examination, as well as hormonal receptors (ER and PR) and epidermal growth factor receptor 2(HER-2), was done by immunohistochemistry [12].

DNA extraction and sodium bisulfite modification

Briefly, extraction of genomic deoxyribonucleic acid (DNA) from breast tumor and adjacent normal breast tissue was done using the Gene JET™ Genomic DNA Purification Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The isolated DNA was evaluated by Nanodrop ND 1000 spectrophotometer (Thermo Scientific, USA) and confirmed by gel electrophoresis running. The ratio of absorbance at 260 nm and 280 nm (A260/A280) was taken to assess the purity of the DNA.

Bisulfite conversion for the extracted DNA was done using the EZ DNA Methylation™ Kit (Zymo Research Corp) according to the manufacturer's instructions. Treatment of DNA with sodium bisulfite converted unmethylated cytosines into uracil, while methylated cytosines remained unchanged. Bisulfite modification step creates sequence differences in the DNA. The converted DNA was then stored at − 20 °C till use. MSP was carried out according to the method described earlier by Herman et al. [13] and performed previously by the author Ramadan et al. [14]. Separate MSP reactions were carried out for both methylated and unmethylated DNA sequences utilizing primer sets specific for each reaction.

The primers used for amplification were:

Forward: 5′-AGTTCGTATAGGCGTTTCGTTC-3′ (methylated), F 5′-AAGTTTGTATAGGTGTTT TGTTTGG-3 (unmethylated).
Backward: F 5′-CTTAACTACAAAAAATTC TTCCGCT-3′ (methylated), F 5′-CTTAACTACAAA AAATTCTTCCACT-3′(unmethylated) [15]

PCR was done by using a total volume of 25 μl (12.5 μl of ZymoTaq™ PreMix, 5 μl of modified bisulfate DNA, 1 μl of each primer, 5.5 μl of nuclease-free H2O). The PCR reaction was performed using thermocycler (Quanta Biotech, UK) under the following conditions: initial denaturation at 95 °C for 10 min followed by 35 cycles with denaturation at 95 °C for 45 s, annealing at 57 °C for 30 s, and extension at 72 °C for 45 s, and finally extension at 72 °C for 7 min. The resultant PCR products were identified by electrophoresis on 2% agarose gel and

stained with ethidium bromide for visualization under UV light.

With each run, positive and negative controls were incorporated. Completely unmethylated and methylated human genomic DNA (EpiTect Control DNA (human); Qiagen) were applied respectively for unmethylation- and methylation-positive control, while nuclease-free sterile water and non-bisulfite-modified DNA were employed as negative control. The PCR generated a 188-bp products for both methylated and unmethylated DNA.

Amplification was successful if a band appeared with the same size as that of the control (100 bp). If amplification was successful in the unmethylated run and failed in the methylated run, this was considered as the unmethylated status (UU genotype) of the *YAP*1 promoter. If the reverse occurred (successful amplification in the methylated run and failed in the unmethylated run), that was hypermethylation (MM genotype). A successful amplification in both runs denoted hemimethylation (MU genotype), and this could suggest methylation heterogeneity where methylation is not inclusive of all CpG sites between the primers, hereby allowing amplification with both primer sets. An aberrant methylation was considered in hypermethylation and hemimethylation situations. All tissue samples were processed and analyzed in a blinded manner. Methylation status was further correlated with clinicopathological criteria.

Statistics

We compared *YAP*1 methylation frequency in tumor and non-tumor breast tissue. MSP results were analyzed as a dichotomous variable based on the occurrence of methylation. The methylation status of *YAP*1 and clinicopathological data were analyzed with the Pearson chi-square test, Fisher's exact test, and Monte Carlo test. The diagnostic performance of *YAP*1 methylation was evaluated. SPSS version 20 was applied, and a p value < 0.05 was considered statistically significant. Each p value was statistically adjusted with the Bonferroni correction.

Results

The study included 50 paired tumor and adjacent normal breast tissues. The participants had a mean age of 46.6 ± 8.45 years. The demographic, biochemical, and clinical parameters of the participants are indicated in Table 1.

Promoter aberrant methylation of the *YAP*1 gene in BC tumor tissue was found in 34 of 50 (68%), while adjacent normal tissue showed methylation in 4 of 50 (8%). The frequency of methylation of the *YAP*1 gene was significantly higher in BC tissue compared to normal breast tissue with ($p < 0.001$).

The analytic results of the correlation between clinicopathological characteristics and methylation status of *YAP*1 gene showed that the aberrant methylation pattern was independent of the age, body mass index, menopausal status, histopathological type, tumor size, axillary lymph node involvement, and serum CA15–3 ($p > 0.05$). However, methylation status was significantly correlated with tumor stage ($p < 0.001$) (Table 1). *YAP*1 methylation showed high accuracy (92%) in discriminating between early and late BC (stage II vs stages II and IV) (Table 2). Although methylation status was significantly correlated with metastasis ($p = 0.02$), it showed low accuracy (52%) in distinguishing those with and without metastasis (Table 3).

Discussion

It is known that YAP1 regulates the downstream effect of Hippo signaling. YAP1 regulates organ size by controlling cellular proliferation and survival. Kinase-mediated phosphorylation of YAP1 promotes its sequestration from the nucleus to the cytoplasm, with subsequent downregulation of its target genes [8, 9].

In the present study, we investigated the usefulness of *YAP*1 as a biomarker in BC. Our results demonstrated that *YAP*1 methylation was significantly higher in BC tissue compared to normal breast tissue and showed a significant correlation with tumor stage. Moreover, the methylation status had good diagnostic performance (accuracy of 92%) in differentiating between early and late stages. Although methylation profile was significantly associated with metastasis and showed 100% sensitivity and negative predictive value, the specificity and positive predictive value were low. This may be attributed to the relatively small percent (10 cases) of participants with metastasis.

The relation of YAP1 with BC was previously examined by immunohistochemical assay, Western blotting, and by real-time quantitative PCR. Different expression levels as well as different subcellular localization were observed [16–19]. The general conclusion of several reports was the association of dysregulation of YAP1 with tumor aggressiveness in BC [20, 21].

In accordance with our findings, Real et al., by using MSP, reported methylation in 70.8% of the studied BC tissue samples. They found a significantly high correlation between *YAP* hypermethylation and *YAP* immunohistochemical expression. Although 84.13% cases of advanced BC stages III and IV had *YAP* promoter methylation and subsequent protein loss, they did not prove any significant association between *YAP* methylation and clinicopathological criteria [15].

Table 1 Correlation analysis of *YAP1* promoter methylation with different variables

Variable	Total *n* (%)	Unmethylated *N* = 16 (%)	Methylated *N* = 34 (%)	Test of Significance	*p* value
Age (years)					
≤ 50	31 (62)	12 (38.7)	19 (61.3)	$\chi^2 = 1.688$	0.194
˃ 50	19 (38)	4 (21.1)	15 (78.9)		
BMI (kg/m^2)					
≤ 25	14 (28)	6 (42.9)	8 (57.1)	$\chi^2 = 1.053$	$^{FE}p = 0.330$
˃ 25	36 (72)	10 (27.8)	26 (72.2)		
Menopausal state					
Premenopausal	17 (34)	4 (23.5)	13 (76.5)	$\chi^2 = 0.849$	0.357
Postmenopausal	33 (66)	12 (36.4)	21 (63.6)		
Histopathological type					
Ductal	34 (68)	12 (35.3)	22 (64.7)	$\chi^2 = 0.534$	$^{MC}p = 0.884$
Lobular	12 (24)	3 (25)	9 (75)		
Others	4 (8)	1 (25)	3 (75)		
Tumor stage					
I				$\chi^2 = 34.124$*	< 0.001*
II	18 (36)	15 (83.3)	3 (16.7)		
III	22 (44)	1 (4.5)	21 (95.5)		
IV	10 (20)	0 (0)	10 (100)		
Tumor size					
≤ 2 cm	3 (6)	2 (66.7)	1 (33.3)	$\chi^2 = 1.763$	$^{FE}p = 0.237$
> 2 cm	47 (94)	14 (29.8)	33 (70.2)		
Axillary lymph node involvement					
Yes	47 (94)	14 (29.8)	33 (70.2)	$\chi^2 = 1.763$	$^{FE}p = 0.237$
No	3 (6)	2 (66.7)	1 (33.3)		
No	40 (80)	16 (40)	24 (60)		
Estrogen receptor status (ER)					
Positive	42 (84)	13 (31)	29 (69)	$\chi^2 = 0.132$	$^{FE}p = 0.699$
Negative	8 (16)	3 (37.5)	5 (62.5)		
Progesterone receptor status (PR)					
Positive	43 (86)	14 (32.6)	29 (67.4)	$\chi^2 = 0.044$	$^{FE}p = 1.000$
Negative	7 (14)	2 (28.6)	5 (71.4)		
HER2 expression status					
Overexpression	16 (32)	2 (12.5)	14 (87.5)	$\chi^2 = 4.112$*	0.043*
Non-overexpression	34 (68)	14 (41.2)	20 (58.5)		
CA15–3 (U/ml)					
Mean ± SD	37.49 ± 28.17	28.78 ± 12.94	41.59 ± 32.37	$t = 1.520$	0.135
Median (min–max)	34 (11–190)				

χ^2 chi-square test, *FE* Fisher's exact, *MC* Monte Carlo, *t* Student's *t* test, *p p* value for comparing between the two groups
*Statistically significant at $p \leq 0.05$

The function of YAP1 on being a tumor suppressor or oncogene is quite controversial. Overexpression of *YAP*1 was observed in lung [22], esophageal [23], and ovarian tumors [24]. The assumption that YAP1 may act as a tumor suppressor was based on the finding that human BC tissue exhibits decreased *YAP*1 expression compared with normal breast tissue [17]. Moreover, knockdown of *YAP*1 in BC cell line and in mouse model demonstrated increased cellular migration and tumor invasiveness [25]. On the contrary, other studies advocated the oncogenic

Table 2 Diagnostic performance of methylation to differentiate early and late tumor stages

	Tumor stage				Sensitivity	Specificity	PPV	NPV	Accuracy
	Early ($n = 18$)		Late ($n = 32$)						
	n	%	n	%					
Methylation									
Unmethylated	15	83.3	1	3.1	96.88	83.33	91.18	93.75	92.0
Methylated	3	16.7	31	96.9					
χ^2 (p)	34.059*(< 0.001*)								

χ^2 chi-square test, *PPV* positive predictive value, *NPV* negative predictive value
*Statistically significant at $p \leq 0.05$

potentials of YAP1, since its overexpression in BC cell line [26] and in mice xenografts [18] increased proliferation and tumorigenesis.

Numerous mechanisms were postulated to explain the relation with BC tumorigenesis. One of which was reported by Shen et al. [27]; their findings showed that YAP1 enhanced tumor invasiveness by modulating thrombospondin 1 transcription, thus leading to focal adhesion kinase phosphorylation in a TEAD-dependent manner. Wang et al. showed that YAP1 promotes metastasis of BC by inhibiting the transcription of growth differentiation factor-15 which is a divergent element in the transforming growth factor superfamily [21].

We studied the relation of *YAP1* methylation with the expression of hormone receptors, and we found a mild significant association with HER2 expression ($p = 0.043$), but no association was observed with ER or PR status. On the other hand, Kim et al. [28] reported that hyper-activation of *YAP*1 in BC tissues was associated directly to the PR status and inversely to HER2 and Ki67 levels. Others described the relation between reduced *YAP*1 expression and decreased recurrence-free survival in luminal cancers [20, 29]. This emphasizes the assumption that, at least in triple-negative BC, YAP1 may possess an oncogenic character. It is plausible that YAP1 acts differently in distinct BC subtypes.

Many laboratory methodologies exist for DNA methylation determination. However, the majority of the methods depend on bisulfite conversion of DNA. Then, the modified DNA is subsequently incorporated to additional molecular techniques; PCR, microarray technology, mass spectrometry or next-generation sequencing. The choice of the appropriate analytical methodology depends on the nature and number of the samples, information required, and cost. MSP is considered a quite sensitive and cost-effective tool without the need for expensive instruments, thus making it applicable to most clinical laboratories [2].

Based on the association of YAP1 with cancer proliferation and drug resistance, there were several trials to identify therapeutic small molecule inhibitors of YAP1 [30, 31]. Verteporfin (VP), a FDA-approved photosensitizing agent used in treatment of neo-vascular macular degeneration, blocks YAP-TEAD binding. Liu-Chittenden et al reported thatVP inhibited liver overgrowth resulting from the overexpression of *YAP*1 by silencing Hippo kinase components [32]. Surprisingly, statins were reported to have inhibitory effects on YAP1 by promoting its cytoplasmic sequestration and degradation [33]. Studies in animal models of cancers indicate that statins have tumor suppressor effects. This was ascertained in clinical studies showing the negative association of statins with cancer occurrence or survival [34].

Certain points are worth mentioning such as the selection of CpG containing regions with respect to differences observed in methylation levels of different locations. Further large-scale studies on different population in addition to validation with different methods,

Table 3 Diagnostic performance of methylation in relation to metastasis

	Metastasis				Sensitivity	Specificity	PPV	NPV	Accuracy
	No metastasis ($n = 40$)		Metastasis ($n = 10$)						
	n	%	n	%					
Methylation									
Unmethylated	16	40.0	0	0.0	100.0	40.0	29.41	100.0	52.0
Methylated	24	60.0	10	100.0					
χ^2(^{FE}p)	5.882*(0.020*)								

FE Fisher's exact test, *PPV* positive predictive value, *NPV* negative predictive value
*Statistically significant at $p \leq 0.05$

and functional analyses of methylation profile are required in order to determine if the specific methylation patterns are exclusively associated with BC.

Conclusion

*YAP*1 methylation was significantly higher in BC tissue compared to normal breast tissue and showed a significant correlation with tumor stage. Moreover, the methylation status had good diagnostic performance in discriminating early and late stages. Our study pinpoints the value of promoter methylation of *YAP1* in BC particularly in view of its future therapeutic potentials.

Abbreviations

BC: Breast cancer; BRCA1: Breast cancer gene; DNA: Deoxyribonucleic acid; MSP: Methylation-specific polymerase chain reaction; PARP: Poly ADP ribose polymerase; TEAD: TEA domain/Transcription Enhancer Factor; VP: Verteporfin; YAP: Yes-associated protein

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Authors' contributions

Author RR conceived of the presented idea. Authors RR and EA developed the study design and criteria of participants' selection. Authors EA and EM collected the specimens. Author RR performed the genetic analysis. All authors performed the statistical analysis, discussed the results, and contributed to the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data are presented in the main paper.

Ethics approval and consent to participate

The study was approved by the Ethical Committee of Medical Research Institute, Alexandria University (IORG#: IORG008812). All subjects have signed informed written consent and agreed on the research purposes of clinical data and samples of our study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H (2014) Cancer incidence in Egypt: results of the national population-based cancer registry program. J Cancer Epidemiol 2014:437971
2. Paska AV, Hudler P (2015) Aberrant methylation patterns in cancer: a clinical view. Biochem Med (Zagreb) 25:161–176
3. Horvath S, Zhang Y, Langfelder P, Kahn RS, Boks MP, van Eijk K et al (2012) Aging effects on DNA methylation modules in human brain and blood tissue. Genome Biol 13:R97
4. Marzese DM, Hoon DS (2015) Emerging technologies for studying DNA methylation for the molecular diagnosis of cancer. Expert Rev Mol Diagn 15: 647–664
5. Wong EM, Southey MC, Fox SB, Brown MA, Dowty JG, Jenkins MA et al (2011) Constitutional methylation of the BRCA1 promoter is specifically associated with BRCA1 mutation associated pathology in early-onset breast cancer. Cancer Prev Res 4:23–33
6. Cai F, Ge I, Wang M, Biskup E, Lin X, Zhong X (2014) Pyrosequencing analysis of BRCA1 methylation level in breast cancer cells. Tumour Biol 35: 3839–3844
7. Overholtzer M, Zhang J, Smolen GA, Muir B, Li W, Sgroi DC et al (2006) Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon. Proc Natl Acad Sci U S A 103:12405–12410
8. Moroishi T, Hansen CG, Guan KL (2015) The emerging roles of YAP and TAZ in cancer. Nat Rev Cancer 15:73–79
9. Totaro A, Panciera T, Piccolo S (2018) YAP/TAZ upstream signals and downstream responses. Nat Cell Biol 20:888–899
10. Hansen CG, Moroishi T, Guan KL (2015) YAP and TAZ: a nexus for Hippo signaling and beyond. Trends Cell Biol 25:499–513
11. Greene FL, Page DL, Fleming ID (eds) (2002) AJCC cancer staging manual, 6th edn, p 435
12. Gown AM (2008) Current issues in ER and HER2 testing by IHC in breast cancer. Mod Pathol 21:S8–S15
13. Herman JG, Graff JR, Myöhänen S, Nelkin BD, Baylin SB (1996) Methylation specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci U S A 93:9821–9826
14. Ramadan RA, Zaki MA, Awad AM, El-Ghalid LA (2015) Aberrant methylation of promoter region of SPINT2/HAI-2 gene: an epigenetic mechanism in hepatitis C virus-induced hepatocarcinogenesis. Genet Test Mol Biomarkers 19:399–404
15. Real SAS, Parveen F, Rehman AU, Khan MA, Deo SVS, Shukla NK et al (2018) Aberrant promoter methylation of YAP gene and its subsequent downregulation in Indian breast cancer patients. BMC Cancer 18:711
16. Zhao B, Tumaneng K, Guan KL (2011) The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal. Nat Cell Biol 13:877–883
17. Tufail R, Jorda M, Zhao W, Reis I, Nawaz Z (2012) Loss of yes-associated protein (YAP) expression is associated with estrogen and progesterone receptor negativity in invasive breast carcinomas. Breast Cancer Res Treat 131:743–750
18. Wang X, Su L, Ou Q (2012) Yes-associated protein promotes tumour development in luminal epithelial derived breast cancer. Eur J Cancer 48: 1227–1234
19. Kim SK, Jung WH, Koo JS (2014) Yes-associated protein (YAP) is differentially expressed in tumor and stroma according to the molecular subtype of breast cancer. Int J Clin Exp Pathol 7:3224–3234
20. Kim HM, Jung WH, Koo JS (2015) Expression of yes-associated protein (YAP) in metastatic breast cancer. Int J Clin Exp Pathol 8:11248–11257
21. Wang T, Mao B, Cheng C, Zou Z, Gao J, Yang Y et al (1864) YAP promotes breast cancer metastasis by repressing growth differentiation factor-15. Biochim Biophys Acta 2018:1744–1753
22. Wang Y, Dong Q, Zhang Q, Li Z, Wang E, Qiu X (2010) Overexpression of yes-associated protein contributes to progression and poor prognosis of non-small-cell lung cancer. Cancer Sci 101:1279–1285
23. Muramatsu T, Imoto I, Matsui T, Kozaki K, Haruki S, Sudol M et al (2011) YAP is a candidate oncogene for esophageal squamous cell carcinoma. Carcinogenesis 32:389–398
24. Zhang X, George J, Deb S, Degoutin JL, Takano EA, Fox SB et al (2011) The Hippo pathway transcriptional co-activator, YAP, is an ovarian cancer oncogene. Oncogene 30:2810–2822
25. Yuan M, Tomlinson V, Lara R, Holliday D, Chelala C, Harada T et al (2008) Yes-associated protein (YAP) functions as a tumor suppressor in breast. Cell Death Differ 15:1752–1759
26. Zhi X, Zhao D, Zhou Z, Liu R, Chen C (2012) YAP promotes breast cell proliferation and survival partially through stabilizing the KLF5 transcription factor. Am J Pathol 180:2452–2461
27. Shen J, Cao B, Wang Y, Ma C, Zeng Z, Liu L et al (2018) Hippo component YAP promotes focal adhesion and tumour aggressiveness via transcriptionally activating THBS1/FAK signalling in breast cancer. J Exp Clin Cancer Res 37:175
28. Kim B, Myung SJ (2018) Clinical implications of the Hippo-YAP pathway in multiple cancer contexts. BMB Rep 51:119–125

29. Cao L, Sun PL, Yao M, Jia M, Gao H (2017) Expression of YES-associated protein (YAP) and its clinical significance in breast cancer tissues. Hum Pathol 68:166–174
30. Guo L, Teng L (2015) YAP/TAZ for cancer therapy: opportunities and challenges (review). Int J Oncol 46:1444–1452
31. Song R, Gu D, Zhang L, Zhang X, Yu B, Liu B et al (2018 Aug 3) Functional significance of Hippo/YAP signaling for drug resistance in colorectal cancer. Mol Carcinog. https://doi.org/10.1002/mc.22883
32. Liu-Chittenden Y, Huang B, Shim JS, Chen Q, Lee SJ, Anders RA et al (2012) Genetic and pharmacological disruption of the TEAD-YAP complex suppresses the oncogenic activity of YAP. Genes Dev 26:1300–1305
33. Sorrentino G, Ruggeri N, Specchia V, Cordenonsi M, Mano M, Dupont S et al (2014) Metabolic control of YAP and TAZ by the mevalonate pathway. Nat Cell Biol 16:357–366
34. Gronich N, Rennert G (2013) Beyond aspirin-cancer prevention with statins, metformin and bisphosphonates. Nat Rev Clin Oncol 10:625–642

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