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Yes-associated protein (YAP) functions as a tumor suppressor in breast

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Yes-associated protein (YAP) has been shown to positively regulate p53 family members and to be negatively regulated by the *AKT* proto-oncogene product in promoting apoptosis. On the basis of this function and its location at 11q22.2, a site of frequent loss of heterozygosity (LOH) in breast cancer, we investigated whether YAP is a tumor suppressor in breast. Examination of tumors by immunohistochemistry demonstrated significant loss of YAP protein. LOH analysis revealed that protein loss correlates with specific deletion of the YAP gene locus. Functionally, short hairpin RNA knockdown of YAP in breast cell lines suppressed anoikis, increased migration and invasiveness, inhibited the response to taxol and enhanced tumor growth in nude mice. This is the first report indicating YAP as a tumor suppressor, revealing its decreased expression in breast cancer as well as demonstrating the functional implications of YAP loss in several aspects of cancer signaling.
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Many of the genetic changes that contribute to the risk of developing sporadic breast cancer are unknown.[1] Previous studies indicated that a tumor suppressor may reside at 11q22–23, a site of frequent loss of heterozygosity (LOH) in sporadic breast cancer.[2–6] One of the genes located at this locus is *Yes-associated protein (YAP)*. YAP was originally cloned as a partner of Yes protein tyrosine kinase,[7] binding at the SH3 domain of Yes but has since been shown to bind to a number of signaling proteins. YAP has been reported to interact with p53-binding protein-2,[8] since shown to be ASPP2, an important regulator of the apoptotic activity of p53.[9] We and others have shown that YAP binds to the p53 family member p73 and is critical for DNA damage induced in breast cancer cells as well as in certain types of neuronal apoptosis.[10–13] More recently, YAP has also been shown to stabilize the p73 protein post-translationally.[14,15] Binding of YAP to p73 is attenuated by phosphorylation of YAP by Akt.[10] As YAP positively regulates members of the p53 family of tumor suppressors and is negatively regulated by the pro-survival *AKT* proto-oncogene product, we assessed whether YAP is the potential tumor suppressor gene located at the 11q22 locus.

Results

YAP protein expression is decreased or lost in breast cancers. We examined the expression of YAP protein in normal breast ($n=20$), benign hyperplasia ($n=18$), preinvasive ductal carcinoma *in situ* (DCIS) ($n=33$) and in invasive ($n=101$) breast cancer by immunohistochemistry (IHC). We selected tumors of luminal origin as deletion around 11q22–23 is more frequent in this subtype.[16,17] Normal breast exhibited strong nuclear expression of YAP in myoepithelial cells with weaker cytoplasmic expression of YAP in the luminal epithelial cells (Figure 1a). In hyperplasia (Figure 1b), strong nuclear YAP immunoreactivity was maintained in myoepithelial cells, with the proliferating luminal population displaying a mixed pattern of nuclear reactivity and cells negative for YAP. This is in keeping with the mixed myoepithelial/luminal proliferation characteristic of benign hyperplasia, though it indicates early loss of luminal YAP. Therefore, loss of YAP expression may be an early and primary event in breast tumorigenesis. In DCIS (Figure 1c and d), again nuclear YAP was maintained in myoepithelial cells. However, the transformed luminal cells were negative

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Abbreviations: DCIS, ductal carcinoma *in situ*; IF, immunofluorescence; IHC, immunohistochemistry; IDC, infiltrating ductal carcinoma; LOH, loss of heterozygosity; shRNA, short hairpin RNA; YAP, Yes-associated protein

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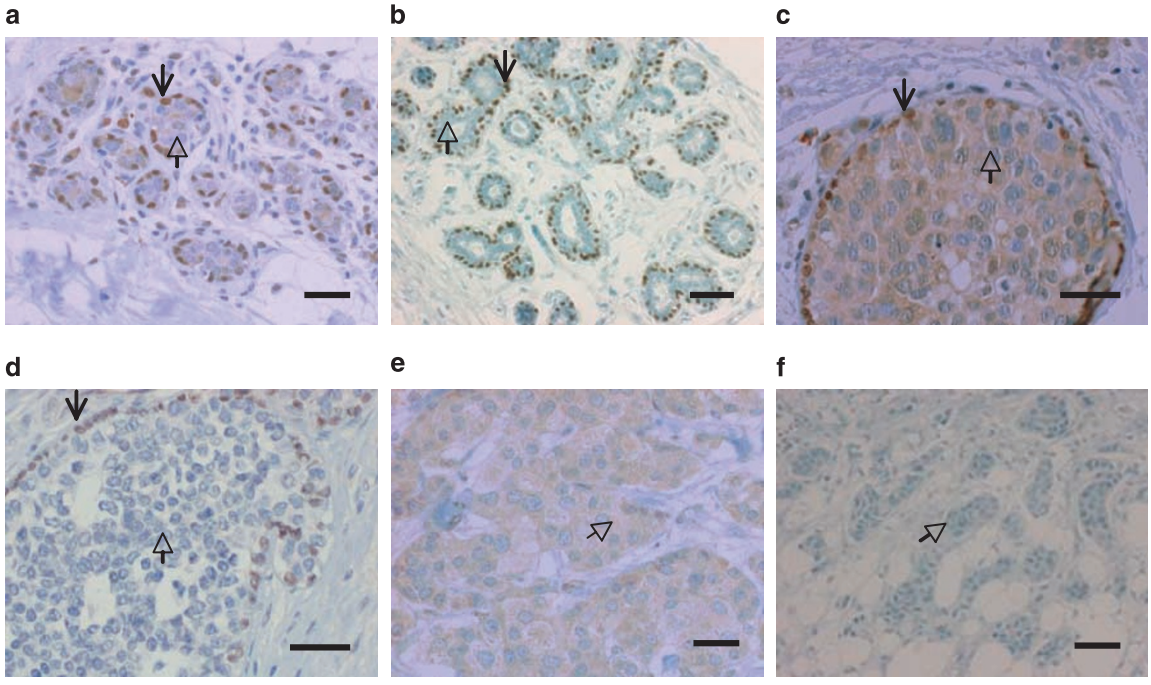

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Histology	sample (n)	YAP expression: positive (n)	YAP expression: negative (n)	YAP expression: negative (%)	p value (Fisher's exact test)
	134	49	85	63.4	
DCIS	33	12	21	63.6	0.5896
IDC	101	37	64	63.4	
IDC	101	37	64	63.4	
I	9	5	4	44.4	0.6955
II	26	8	18	69.2	
III	66	24	42	63.6	

Figure 1 YAP protein expression in normal breast and breast cancer. (**a**) Example of IHC analysis of normal breast tissue using YAP antibody. YAP is positive in nucleus of all myoepithelial cells as indicated by dark arrow. YAP is positive in cytoplasm of luminal cells as indicated by light arrow. (**b**) Representative example of IHC of hyperplasia using YAP antibody. YAP is positive in nucleus of all myoepithelial cells as indicated by dark arrow. YAP is negative in luminal cells as indicated by light arrow. (**c**) Representative example of IHC observed in DCIS using YAP antibody. YAP is positive in nucleus of all myoepithelial cells as indicated by dark arrow. YAP is weakly positive in luminal cells as indicated by light arrow. (**d**) Representative example of IHC observed in DCIS using YAP antibody. YAP is positive in nucleus of all myoepithelial cells as indicated by dark arrow. YAP is negative in luminal cells as indicated by light arrow. (**e**) Representative example of IHC observed in IDC using YAP antibody. YAP is weakly positive in cytoplasm of luminal cells as indicated by light arrow. (**f**) Representative example of IHC observed in IDC using YAP antibody. YAP is negative in luminal cells as indicated by light arrow. Bar, 50 μM (**a**–**f**). (**g**) Summary of IHC of YAP in stages of breast carcinogenesis

for YAP in 63.6% (21 of 33) of the cases (Figure 1d), with the remaining 36.4% (12 of 33) cases showing a variable intensity of cytoplasmic immunoreactivity (Figure 1c). In infiltrating ductal carcinomas, 63.4% (64 of 101) of cases showed loss of YAP expression (Figure 1e and f). Analysis of YAP expression in relation to conventional prognostic indices of breast carcinoma showed no significant correlation with tumor grade, though there was a trend toward greater frequency of loss in the higher grade tumors, with 55.6% (5 of 9) grade I, 30.8% (8 of 26) grade II and only 36.4% (24 of 66) grade III retaining YAP expression within the neoplastic population (Figure 1g). There was no relationship between YAP expression and ER, HER2 or lymph node status (Supplementary Table 1).

Given that YAP has been shown to interact with the p53 family and p53 mutation itself occurs in breast cancer, we investigated whether there was a correlation between loss of YAP and p53 mutations. Specifically, we wanted to see if loss of YAP was subsequent to and possibly a result of p53 mutation. IHC staining for p53 and YAP protein indicated that there was no correlation between YAP and accumulation of mutant p53. In fact, we demonstrated that loss of YAP occurred earlier in the pathogenesis of the disease than accumulation of mutant p53 (Supplementary Figure 1 and Supplementary Table 1). There was also no correlation between YAP and p63 expressions (Supplementary Figure 1 and Supplementary Table 1).

YAP protein loss is correlated with deletion of *YAP* gene locus. To elucidate the mechanism of loss of YAP protein expression in breast cancer, we wished to determine whether this protein loss was caused by deletion of the *YAP* gene. Though frequent LOH in this region has been reported for breast cancer,[2–6] particularly of the luminal subtype, we

performed LOH analysis employing microsatellite marker D11S1339, specific for the actual *YAP* locus (Figure 2a). We detected LOH at D11S1339 (Figure 2b) in 33.3% (9/27) of cases tested in a sample set of breast tumors in which YAP loss by IHC was 50% (13/26). Further analysis of the cases exhibiting LOH showed that 77.8% of cases exhibiting LOH also showed loss of YAP by IHC (7/9) whereas about half of samples without LOH showed YAP protein loss (Figure 2c). Thus, microsatellite marker analysis for *YAP* shows deletion of the *YAP* locus, consistent with the LOH previously seen in this region in breast cancer and in accordance with our findings of YAP protein loss in breast cancer.

To rule out other possible mechanisms for downregulation of YAP in breast cancer, we analyzed the promoter region for epigenetic silencing by promoter methylation and also sequenced each of the exons to locate possible mutations. Neither methylation nor mutation was detected and thus there was nothing in our studies to support either an epigenetic or sequence mutation basis for *YAP* downregulation in breast cancer (Supplementary Figure 2, full analysis provided in Supplemental materials).

Having established that YAP was frequently deleted in luminal-derived breast cancers, we next characterized its tumor suppressor functions both *in vitro* and *in vivo*. To ensure against possible spurious effects of YAP loss in one cell line and also to account for the heterogeneity of luminal breast cancers, we assayed a number of breast cancer cell lines of luminal origin, including T47D, BT474 and MCF-7, as well as the more mesenchymal MDA-MB-231 cell line. We generated stable cell lines transduced with either a pRetroSuper-derived short hairpin RNA (shRNA) vector targeting YAP (pRS-IRES-GFP-YAP) or a control virus (pRS-IRES-GFP). YAP protein expression was effectively suppressed in the stable cell lines, as shown as an example for MDA-MB-231 cells by quantitative real-time PCR (q-PCR) (Supplementary Figure 3a), western blot (Supplementary Figure 3b) and immunofluorescence (IF) (Supplementary Figure 3c). YAP expression by western blot is also shown for control and shRNA stable T47D and BT474 cells (Supplementary Figure 3d).

YAP loss protects cells from anoikis. YAP loss by siRNA has been shown by many groups to protect breast cancer cells from DNA damage-mediated apoptosis.[10,12,13] Here, we examined the effect of YAP loss on anoikis, a program of detachment-induced cell death critical to breast cancer progression.[18] YAP knockdown rendered BT474 and MDA-MB-231 cells significantly resistant to anoikis at 24, 48 and 72 h in suspension, and T47D cells significantly resistant at 48 and 72 h in suspension (Figure 3a). We also saw protection from anoikis in cells transfected with two separate siRNA oligonucleotides, targeting different regions on the YAP sequence, indicating that the result of the YAP siRNA was specific and not an off-target effect (Supplementary Figure 4). After 48 h of culture in suspension, cells were re-plated and resultant colonies scored. YAP knockdown cells exhibited not only decreased cell death from anoikis but also increased colony formation (Figure 3b). These results were corroborated in T47D cells in which YAP knockdown also significantly promoted colony formation in soft agar (Figure 3c), suggesting that besides protection from anoikis, YAP loss also promotes anchorage-independent growth.

Breast cancer cells with YAP silencing exhibit increased migration and invasion

To further analyze the role of YAP in tumorigenesis, we conducted cell migration and invasion assays of the YAP- and

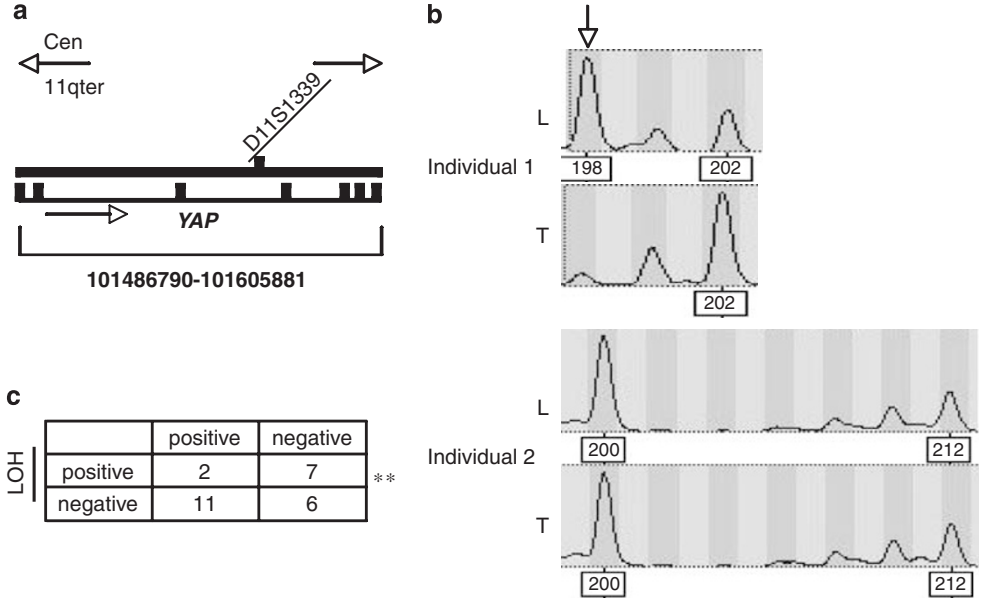


Figure 2 Deletion of YAP gene in breast cancer. (**a**) Contig map showing the locus of YAP gene and the location of microsatellite marker D11S1339. Map not drawn to scale. (**b**) Sample case representative individual 1 shows complete loss of an allele from the D11S1339 marker and individual 2 shows retention of heterozygosity at D11S1339. L = normal lymph node sample; T = tumor sample. (**c**) Comparison of IHC for YAP and LOH status by microsatellite analysis of YAP in representative breast tumor types. $^{**}\chi^2 = 4.25$, $P = 0.039$

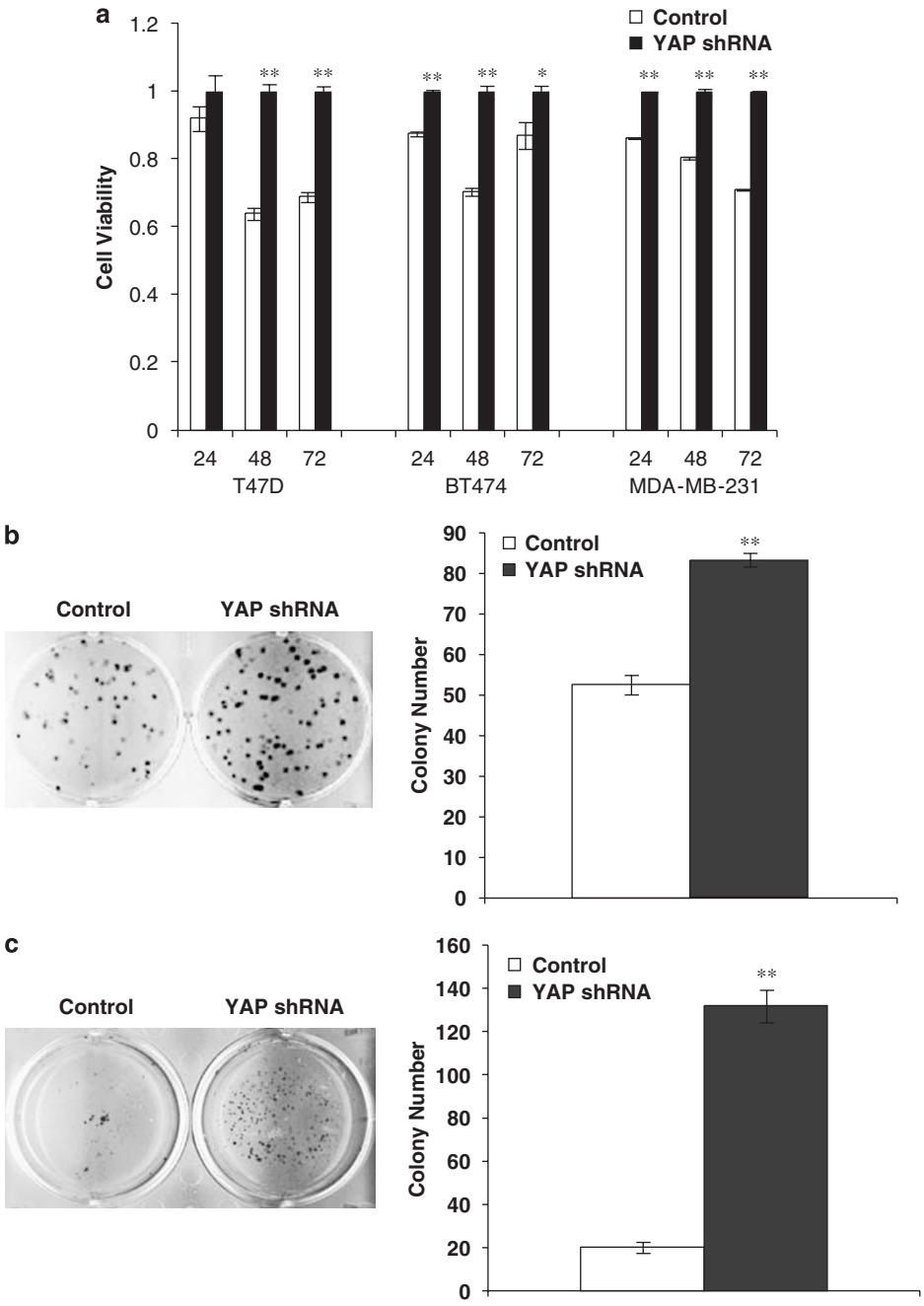


Figure 3 YAP loss protects cells from anoikis and promotes anchorage-independent growth. T47D, BT474 and MDA-MB-231 were stably expressed with either pRS-IRES-GFP (control) or pRS-IRES-GFP-YAP (YAP shRNA). (**a**) Anoikis was induced by culturing in ultra low binding plates and viability measured by MTS in control and indicated YAP shRNA cells at indicated times. T47D 24 h: -1.25, $P=0.279$; T47D 48 h: $^{**}t=-9.49$, $P=0.000$; T47D 72 h: $t=-12.5$, $P=0.000$; BT474 24 h: $^{**}t=-12.3$, $P=0.000$; BT474 48 h: $^{**}t=-15.1$, $P=0.000$; BT474 24 h: $^{**}t=-12.3$, $P=0.000$; BT474 72 h: $^{*}t=-5.81$, $P=0.004$; MDA-MB-231 24 h $^{**}t=-42.2$, $P=0.000$; MDA-MB-231 48 h $^{**}t=-19.3$, $P=0.000$; MDA-MB-231 72 h $^{**}t=-74.9$, $P=0.000$. (**b**) Cells of MDA-MB-231 control and YAP shRNA from culture in ultra low binding plates were re-plated to grow for 3 more days in normal culture; colonies were stained by MTS and quantified. $^{**}P=0.0005$. (**c**) Anchorage-independent growth of T47D control and YAP shRNA cells was looked at using colony formation in soft agar assay. Colonies were stained using MTT as shown, colony number was counted and plotted.$^{**}t=-24.6$, $P=0.000$. $N=3$ for all quantitations. The figures of (**b**) and (**c**) represent one of three independent experiments

control-shRNA vector-transduced MDA-MB-231 cells. Cell migration recorded by time-lapse microscopy showed that cells with YAP suppression migrated farther and in a wider range of direction than control cells, and quantitation of this cellular movement confirmed that loss of YAP significantly enhances cell migration (Figure 4a). We also assessed the effect of YAP knockdown on cellular invasion using the matrigel invasion assay for YAP- and control-shRNA vector-transduced MCF-7 in which YAP loss significantly increased invasion (Figure 4b).

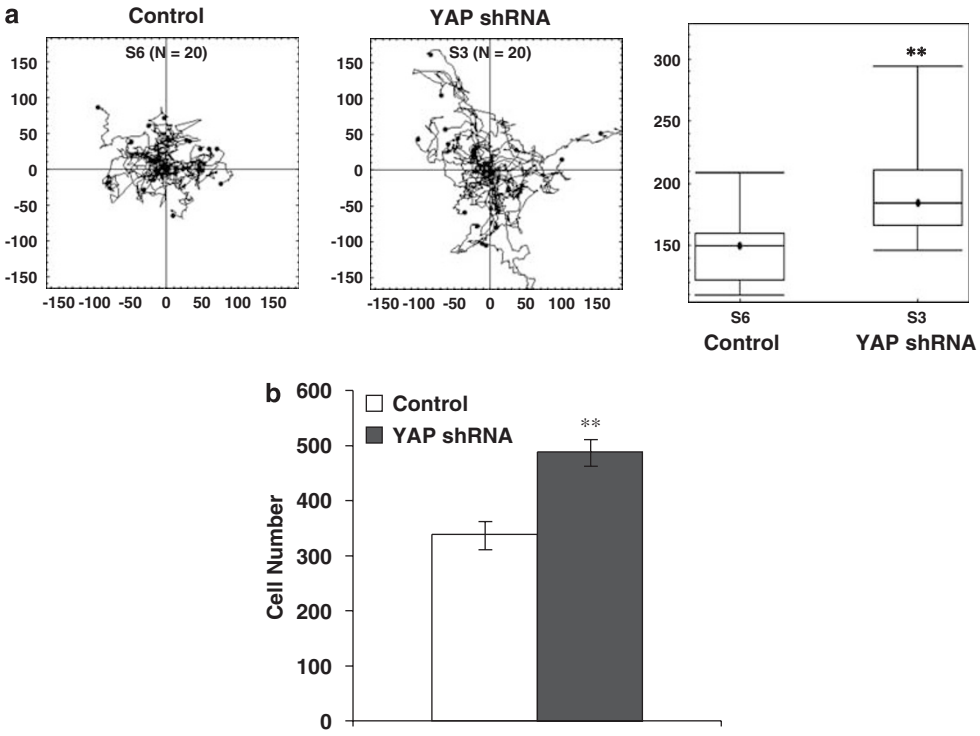


Figure 4 YAP loss promotes cell migration and invasion *in vitro*. (**a**) Migration of control and YAP shRNA MDA-MB-231 cells measured by time-lapse microscopy to track the movement of cells over 24 h and the average movement quantified over 24 h. (**a**) $^{**}P = 0.00198$ (ANOVA). (**b**) Control and YAP shRNA MCF-7 cells were cultured in transwell matrigel and invasive cells stained and quantified, in triplicate. The figure represents one of three independent experiments. $^{**}P = 0.002$

YAP-silenced normal breast epithelia are more resistant to taxol effect on cell cycle. To examine whether the normal untransformed luminal breast epithelial cells were affected by YAP silencing, we assessed response to the microtubule poison, taxol, which is used against luminal breast cancers. Stable YAP knockdown in 1089 normal cells (Supplementary Figure 5c) inhibited their taxol-mediated cell cycle arrest in G2 (Figure 5). Specifically, the G2/G1 ratio in control cells upon taxol treatment was 2.47, whereas the ratio for YAP shRNA cells was 1. Taxol treatment did not result in an appreciable population of cells in sub-G1 population (Figure 5) or apoptosis as measured by MTS (Supplementary Figure 5a) and only in a slight and not significant ($P = 0.1113$) increase in Annexin V-positive cells (Supplementary Figure 5b).

***In vivo* tumor growth is increased with YAP knockdown.** Finally, MDA-MB-231 pRS-IRES-GFP-YAP and control cells were injected into nude mice and the kinetics of tumor growth observed. Tumors were formed earlier and grew faster in mice injected with cells in which YAP was knocked down, compared with controls, demonstrating a significant role of YAP in tumor growth (Figure 6a). Sustained loss of YAP expression in the xenograft tumor was determined by IHC (Figure 6b).

Discussion

This is the first report of YAP as a potential tumor suppressor in breast cancer. Though amplification of 11q22 has been found in 5–10% of human cancer types including oral squamous cell carcinomas, as well as cancers of the pancreas, lung, ovary, esophagus and liver,[19–23] this is a site of frequent LOH in breast cancers. Of interest, we demonstrate profound protein loss of YAP in our patient samples though LOH analysis shows only monoallelic loss. Promoter methylation and mutation analysis ruled out these as possible mechanisms for further loss of *YAP* in breast cancer. We propose that *YAP* is a haploinsufficient tumor suppressor, implying that LOH is sufficient to cause the striking protein loss occurring in breast cancer. This is similar to Tip60, which is also involved in DNA damage, and has recently been shown to be a tumor suppressor in breast where monoallelic loss results in functional loss of protein.[24] Shaul's group as well as we have recently shown that YAP is involved in stabilizing p73 by interfering with E3 ligase-dependent ubiquitylation in a post-translational manner.[14,15] YAP may be similarly regulated and this, coupled with already reduced message, may account for the greater loss of protein than gene expression in our study.

Although YAP was recently shown to have oncogenic function in a model based on the non-transformed mammary myoepithelial-derived cell line, MCF10A, no YAP copy number changes were found in over 100 sporadic cases of human breast cancer in the same report.[25] LOH at 11q22–33 is thought to be more frequent in the luminal subtype and it may be that *YAP* gene expression and subsequent protein levels are different in these, which are the vast majority in breast cancers and the subject of this study, than in the rarer but more aggressive myoepithelial-derived tumors.

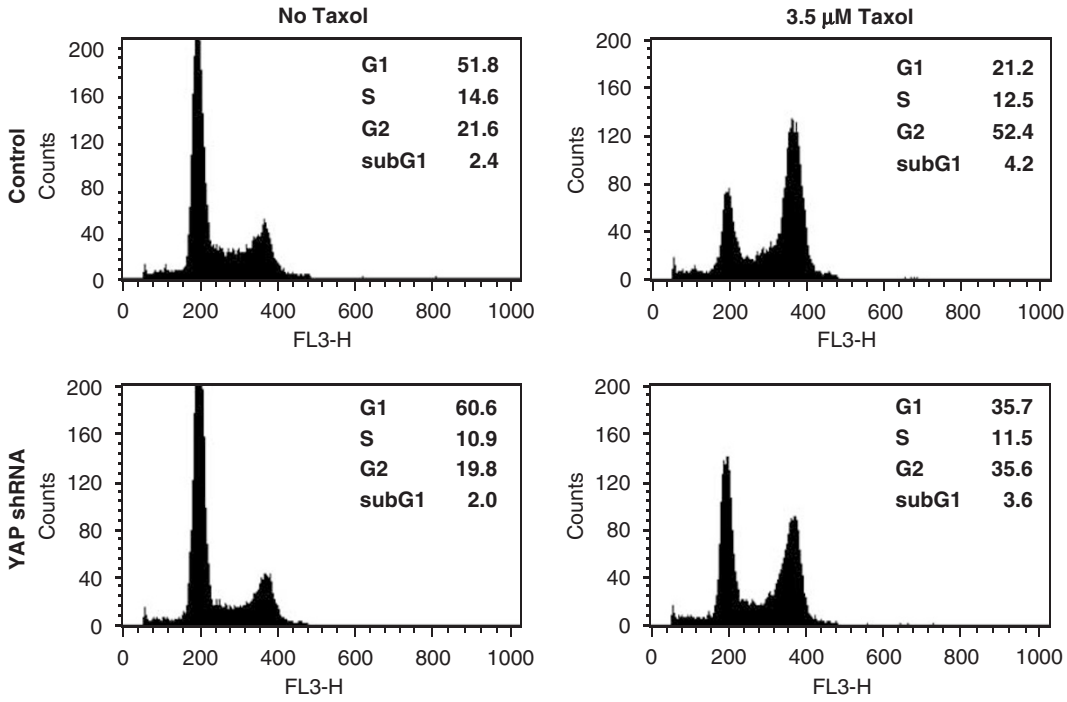
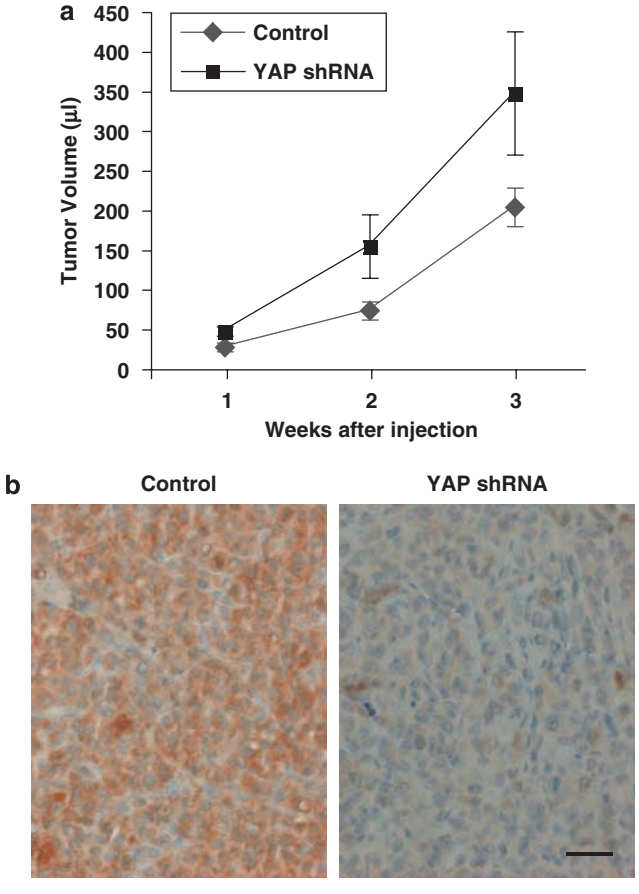


Figure 5 YAP loss decreases response to taxol treatment in normal breast cells. Normal luminal epithelial cell line 1089 control and YAP shRNA cells were untreated or treated with 3.5 μM taxol for 24 h, and cell cycle profiles were analyzed using PI staining. Percentage of cells in G1, S and G2/M and sub-G1 phases of cell cycle are shown in inset



Furthermore, as the subcellular localization of YAP varies between the cell types, it may be that YAP may function differently in them and in the tumors derived from them, as alluded to in recent studies.[26,27] Interestingly, another WW domain-containing protein, WWOX, which also changes subcellular localization, has been implicated as a tumor suppressor and prognostic indicator in breast cancer,[28] indicating that regulation of subcellular localization may be as important as expression level in determining tumor suppressor function.

Functionally, the majority of work on YAP signaling has focused on its role in regulation of transcription factors, notably the p53 family member, p73. Though this and its negative regulation by Akt led us to investigate the role of YAP as a tumor suppressor, YAP also binds many other transcription factors. It may be that the integration of the different transcriptional programs regulated by YAP and the specific effect of YAP loss or overexpression will determine its role as either a tumor suppressor or as an oncogene in tissue and tumor subtypes, consistent with a network view of signaling in which proteins can play diverse roles depending on cellular as well as stimuli context. This was recently demonstrated in two recent reports in which in *Drosophila*, the YAP homolog, yorkie, is crucial for organ size and proliferation downstream of the HIPPO/WTS pathway, but downstream of the

Figure 6 YAP loss promotes tumor progression in mice. (**a**) Tumorigenicity of control and YAP shRNA-transduced MDA-MB-231cells, injected subcutaneously into both flanks of nude mice ($n = 5$), was assessed by tumor volume over indicated time. Error bars represent ± S.E.M. (**b**) YAP expression by IHC in tumors from mice injected by control and YAP shRNA cells

homologous MST2/LATS in human cells, YAP was shown to be pro-apoptotic through p73, consistent with our own findings.[12,29] Notably, the work in the human MTS/LATS pathway showing YAP to be pro-apoptotic was undertaken in breast cells. To add to this complexity, YAP exists in two major isoforms, YAP1 and YAP2, which may differ in expression and transcriptional coactivation of targets.[30] For example, YAP1 is expressed in stem cells and functions more for cell growth in that context.[31] It may be that their relative expression levels may also regulate whether YAP is functioning as a tumor suppressor or oncogene in precancerous cells. A greater understanding of the different functions and mechanisms of regulation of YAP in various cell types will be necessary to further interpret the varied roles of YAP in tumorigenesis.

Materials and Methods

Cell culture. The MDA-MB-231, MCF-7, T47D, BT474 and SKBR-3 human breast cancer cell lines (Cancer Research UK Cell services, Clare Hall, UK) were cultured as described previously.[10] Human primary luminal mammary 1089 cell lines were generated and cultured as previously described.[32] Control and YAP shRNA stable cell lines were made by transduction with either pRS-IRES-GFP or pRS-IRES-GFP-YAP, respectively. Targeting sequence of YAP shRNA was described previously.[10]

IHC. Paraffin sections of normal and tumor sections were subjected to analysis for YAP expression using YAP antibody (H-125; Santa Cruz, 1 : 40), p53 (DO-7; Dako, 1 : 100) and p63 (4A4; Abcam, 1 : 250), and sections were counterstained with hematoxylin. The staining was done on the Ventana Discovery system (USA) as previously described.[33] ERα (Novocastra, 1 : 100) and Her-2 (CB11; Dako, 1 : 100) were demonstrated using pressure-cooking antigen retrieval followed by standard Avidin–Biotin Complex IHC. Images were acquired using a Zeiss Axiophot microscope ($\times$ 40) and Nikon DXM1200 digital camera.

IF staining. IF staining of YAP was performed on a Zeiss LSM 510 Meta confocal microscope with YAP antibody as previously described.[10]

DNA extraction from tissue. DNA was extracted with Dnaeasy Tissue Kit (Qiagen) following the manufacturer's instructions.

Detection of LOH in tumors. We genotyped, in triplicate, 24 matched lymph node and tumor DNA samples, after microdissection to ensure retrieval of tumor tissue only, using the microsatellite marker D11S1139 located within intron 3 of the YAP gene. Briefly, 10 ng of genomic DNA was amplified using oligonucleotide primers, one of which was labeled at the 5′-end with the fluorescent dye FAM. Two microliters of PCR product was combined with 0.02 μl ROX400 marker (ABI) and 8 μl Hi Dye Formamide (ABI) with detection on a 3700 genetic analyzer (ABI). Data were interpreted using Genotyper v 3.7. LOH status was determined both visually and by calculating the peak ratios between the constitutional and tumor alleles.

Anoikis assay. Costar Ultra-low cluster six-well plates (Corning) were used for the anoikis assay. A total of 1×10^6 cells were seeded in a single well of a six-well plate in triplicate for each time point. Cell survival was determined by MTS assay according to the manufacturer's instructions (Promega). Mean value and standard error of sample mean (S.E.M.) were calculated. All assays were done in triplicate in three independent experiments. For transient knockdown of YAP and assessment of anoikis, T47D and BT474 cells were seeded in six-well plates at a density 200 000 per well. The following day, cells were transfected with 20 nM siGENOME Non-Targeting siRNA no 2 (Dharmacon), 20 nM YAP1 ON-TARGET plus Duplex J-012200-08 (siRNA YAP8) (Dharmacon) or 10 nM YAP siRNA 1 (sequence described previously)[10] using Interferin (Polyplus Transfection). After 24 h, the transfected cells were re-plated in triplicate in both Poly-Hema (Sigma)-coated and -uncoated 96-well plates and cell viability measured using an MTS Assay (Promega) 24 and 48 h after plating. The viability of cells transfected with control siRNA, siRNA YAP1 and siRNA YAP8 and plated on Poly-Hema-coated plates was calculated relative to the same cells plated on uncoated plastic. The viability of control siRNA-transfected cells is shown relative to YAP siRNA-transfected cells and the S.D. of the mean was calculated.

Anchorage-independent growth. Cells (1×10^5) were added to 1.5 ml of growth medium with 0.35% agar and layered onto 2 ml of 0.5% agar base in six-well plates. Cells were fed with 2 ml of medium every 3 days for 3 weeks, after which colonies were stained with MTT and photographed. Visible colonies were counted as positive for growth, and mean value and S.E.M. were calculated. Assays were conducted in triplicate in three independent experiments.

Migration assay. Cells were plated at 25% confluence in 96-well plates. Twenty-four hours later, each plate was imaged for 18 h (time-lapse 1 image per 10 min per well) using a motorized-staged environment-controlled Nikon TE2000 microscope and images captured using an Andor IXON camera. Each condition was performed in triplicate. At least 60 cells per condition were tracked and motility of indicated cells was evaluated by interactive tracking using Motion Analysis software (Kinetic Imaging). Individual tracks were analyzed using software Mathematica (Wolfram Research) that calculated mean displacement per 5-min lapse interval for each cell. This value was then converted to displacement per hour, and mean value and S.E.M. were calculated for pooled cells from the two pairs of movies. The significance of the difference was evaluated by analysis of variance. Assays were performed in three independent experiments.

Invasion assays. The Matrigel invasion Kit (BD Biosciences) was used according to the manufacturer's instructions. A total of 50 000 cells in suspension were added to the upper well of invasion chambers in triplicate and incubated at 37°C for 48 h. Cells that had traversed the filter were counted by light microscopy, following removal of surface-adherent cells and staining with Giemsa. Mean invasion cells and S.E.M. were calculated. Assays were performed in triplicate in three independent experiments.

Western blot analysis. Protein extracts were fractionated by SDS-PAGE and transferred to PVDF membrane (Hybond-P; Amersham Pharmacia Co.). Anti-YAP rabbit polyclonal antibody (H-125) (Santa Cruz, 1 : 200) and anti-Actin and anti-PCNA mouse monoclonal antibodies (RMAS; CR-UK, 1 : 1000) were used as indicated. Antibody binding was detected by chemiluminescence (ECL; Amersham Pharmacia Biotech) and the membranes were exposed to X-ray film.

Cell cycle analysis. Cells were harvested and fixed in 75% ice-cold ethanol at 4°C for 2 h. Then, cells were stained with propidium iodide (PI) (Molecular Probes) and analyzed by flow cytometry. Assays were conducted in three independent experiments.

Annexin V. Cells were plated in six-well plates at a density of 150 000 per well. Twenty-four hours later, taxol was added to appropriate wells at a concentration of 3.5 μM and the cells incubated for 24 h. Cells were then assayed for apoptosis using Annexin V Alexa Fluor 488 conjugate and PI according to the manufacturer's protocol (Molecular Probes) and analyzed by flow cytometry. The assay was repeated in three independent experiments.

Tumor growth in mice. Five female BALB/c SCID mice aged 6–8 weeks (Harlan) were used in each group. A total of 1×10^7 MDA-MB-231 pRS-IRES-GFP or pRS-IRES-GFP-YAP cells were mixed 1 : 1 by volume with matrigel (BD Biosciences) per injection. Each mouse was injected in both flanks. Tumor development was measured twice weekly from first appearance and tumor volume was calculated as Volume = (Length $\times$ Width^2 $\times$ 3.1415926)/6.

q-PCR. Quantitative, real-time PCRs were carried out with SYBR Green PCR master mix (Applied Biosystems) and the products detected with the ABI 7700 Sequence Detector (Applied Biosystems). YAP primers sequences are forward 5′-AACTCGGCTTCAGCCATGA-3′ and reverse 5′-GCTACGCAGGGCTAACTC CTGT-3′, and GAPDH primers are described previously.[34]

Statistical analysis. We performed statistical analysis using Student's *t*-test and χ^2 test. Results were considered significant at $P \leqslant 0.05$.

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Supplementary Information accompanies the paper on Cell Death and Differentiation website (http://www.nature.com/cdd)