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Identification of a Tumor Suppressor Relay between the FOXP3 and the Hippo Pathways in Breast and Prostate Cancers

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Abstract

Defective expression of LATS2, a negative regulator of YAP oncoprotein, has been reported in cancer of prostate, breast, liver, brain, and blood origins. However, no transcriptional regulators for the LATS2 gene have been identified. Here we report that spontaneous mutation of the transcription factor FOXP3 reduces expression of the LATS2 gene in mammary epithelial cells. shRNA-mediated silencing of FOXP3 in normal or malignant mammary epithelial cells of mouse and human origin repressed LATS2 expression and increased YAP protein levels. LATS2 induction required binding of FOXP3 to a specific sequence in the LATS2 promoter, and this interaction contributed to FOXP3-mediated growth inhibition of tumor cells. In support of these results, reduced expression and somatic mutations of FOXP3 correlated strongly with defective LATS2 expression in micro-dissected prostate cancer tissues. Thus, defective expression of LATS2 is attributable to FOXP3 defects and may be a major independent determinant of YAP protein elevation in cancer. Our findings identify a novel mechanism of LATS2 downregulation in cancer and reveal an important tumor suppressor relay between the FOXP3 and HIPPO pathways which are widely implicated in human cancer. Cancer Res; 71(6); 2162–71.

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Introduction

Genetic studies in Drosophila have established an important role for the Hippo pathway in regulation of cell proliferation and apoptosis (1–3). Components of the Hippo pathway, including Yap, Lats1/2, and Mst1/2 (Drosophila Yki, Hpo, and Wts homologs, respectively) are highly conserved between Drosophila and human, as the human YAP, LATS2, and MST2 are capable of rescuing the corresponding Drosophila mutants (1, 3). The functional conservation raised the possibility that the Lats1 and Lats2, the mammalian Wts homologs may function as tumor suppressors. In support of this notion, targeted mutation of Lats1 caused soft-tissue tumor in the mice (4). Although Lats2 deletion is embryonic lethal, analysis of the Lats2−/− murine embryonic fibroblast suggests a critical role of Lats2 in genome stability and growth inhibition (5). Recent studies have revealed that LATS2 regulates cellular localization (6, 7) and degradation (8) of YAP protein. Transgenic expression of an active YAP mutant lacking a Lats2 phosphorylate site caused liver cancer (6). The significance of LATS2 in human cancer is supported by widespread downregulation of LATS2 in cancers in breast (9), prostate (10), brain (11), and blood (12). However, genetic lesions that disrupt the LATS2 expression have not yet been identified.

FOXP3 is a newly identified X-linked tumor suppressor gene for both prostate and breast cancers (13, 14). Our recent studies have shown that, as a transcriptional factor, Foxp3 inhibits tumor cell growth by both repressing oncogenes, Erbb2 (14), cMyc (13), and Skp2 (15) and inducing tumor suppressor p21 (16). Here we report that Foxp3 is a direct transcriptional activator for Lats2 in both normal and malignant breast and prostate cells from mouse and human. Mutation or downregulation of Foxp3 decreased Lats2 expression. These data show a functional relay between 2 newly identified tumor suppressor genes.

Materials and Methods

Mice

Rag2−/−Foxp3+/+ and Rag2−/−Foxp3−/− female and male Rag2−/−Foxp3+/+ and Rag2−/−Foxp3−/− male BALB/c mice have been described previously (17). Four-month-old virgin mice were used to analyze the effect of Foxp3 mutation on Lats2 expression and hyperplasia of mammary epithelia. All animal experiments were conducted in accordance with accepted standards of animal care and approved by the institutional Animal Care and Use Committee of University of Michigan.
Cell culture
Breast cancer cell line MCF-7 was purchased from the American Type Culture Collection and immortalized mammary epithelial cell line MCF-10A was obtained from Dr. Ben Margolis (University of Michigan). A Tet-off FOXP3 expression system in the MCF-7 cells has been established previously (14). Cell banks were created after cells were received. Early passages of cells were used for the study. No reauthentification of cells has been done since receipt.

FOXP3 silencing
The human FOXP3 silencing vectors were described previously (16). The mouse Foxp3 shRNA and control lentiviral vectors pLKO.1 were purchased from Open Biosystems.

Western blot
The anti-FOX3 (hFOXY; eBioscience; 1:100), anti-Lats2 (Cell Signaling; 1:1,000), anti-Yap, anti-p-Yap(Cell Signaling) and anti–β-actin (Sigma, 1:3,000) were used as primary antibodies. Anti-rabbit or mouse immunoglobulin G (IgG) horseradish peroxidase–linked secondary antibody at 1:5,000 to 1:10,000 dilutions (Cell Signaling) was used.

Chromatin immunoprecipitation
Chromatin immunoprecipitation (ChIP) was carried out according to the published procedure (16). Briefly, the FOXP3-transfected Tet-off cells were sonicated and fixed with 1% paraformaldehyde. The anti-FOX3, and anti-IgG (Santa Cruz Biotechnology) antibodies were used to pull down chromatin associated with FOXP3. The amounts of the specific DNA fragment were quantitated by real-time PCR and normalized against the genomic DNA preparation from the same cells. The ChIP real-time PCR primers are listed in Supplementary Table S1.

Quantitative real-time PCR
Relative quantities of mRNA expression were analyzed by real-time PCR (ABI Prism 7500 Sequence Detection System; Applied Biosystems). The SYBR (Applied Biosystems) green fluorescence dye was used in this study. The primer sequences are listed in the Supplementary Table S1.

Tumorigenicity assay
TSA cells (10^6 per inoculation) were injected into mammary fat pads of syngeneic BALb/c mice. The tumor volumes are defined as 0.5πr^3, where r is radius. The tumor diameters

Figure 1. Foxp3 induced Lats2 expression in normal and malignant mammary epithelial cells in the mice. A, mouse mammary (top) and prostate (bottom) epithelial cells were isolated from Rag2^−/− Foxp3^+/+ and Rag2^−/− Foxp3^m/m mammary glands or Rag2^−/− Foxp3^−/− and Rag2^−/− Foxp3^m/m prostate. Lats1, Lats2, and Foxp3 mRNA levels were determined by real-time RT-PCR. Data shown were mean ± SD of mRNA levels presented as %GAPDH and have been repeated 3 times. B, Foxp3 regulates Lats2, Yap protein levels, and Yap phosphorylation (Yap-pS127), as shown by Western blot. The results have been repeated 3 times. C, immunohistochemical analysis for Lats2 protein expression in benign mammary tissue from WT and Foxp3 mutant mice, as well as a mammary tumor of Rag2^−/− Foxp3^+/+ origin. D, Foxp3 regulates expression of the Lats2 gene in mouse mammary tumor cell line. Lats2 mRNA in murine mammary tumor cell line TSA transfected with either scrambled or Foxp3 siRNA. Transfected cells were selected by puromycin for 2 weeks after transfection. The results have been repeated 3 times.
were derived from the average of largest diameters in two dimensions.

**Site-directed mutagenesis**

All mutants were generated by using mutagenesis kit from Stratagene (catalog no. 210518). The deleted sequence LATS2 promoter mutants were ATAACAT for B6M, CAGTTGT for B7M, and TGTTTAT for B8M.

**Immunohistochemistry**

Immunohistochemistry was done by the avidin–biotin complex method. Expression of FOXP3 in human breast cancer or normal tissue samples was determined by immunohistochemistry, as described (13). The rabbit anti-Lats2 monoclonal antibody (Cell Signaling; 1:200), and biotinylated goat anti-mouse IgG were obtained from Santa Cruz and used at 1:200. FOXP3 and Lats2 staining of tissue microarray (TMA) samples (US Biomax, Inc.) were scored double blind.

**DNA sequencing for human YAP gene in prostate cancer samples**

To test whether human YAP is somatically mutated in primary prostate cancer samples to gain resistance to regulation by LATS, we sequenced exons 2 and 6 of human YAP, which encodes amino acid sequence encompassing S127 and S347 (S381) sites, respectively. DNA were prepared from microdissected normal and cancerous prostate tissues from the same patients. The genomic DNA were amplified by PCR by using a forward primer (AACCTGTGTTCTCCAGTGTCG) and a reverse primer (ACCCGGCCAATCCATGATAT) for exon 6. The PCR products were sequenced. When ambiguous results were obtained, the DNA will be cloned and sequenced. At least 5 clones were analyzed per sample.

**Statistical analysis**

Data are shown as mean ± SD. Statistical analysis was performed with Student’s t test. ANOVA tests were used to analyze data with more than 2 groups. Chi-square test was used to determine statistical significance of relationship between the expression of FOXP3 and LATS2.

**Results**

**Requirement for Foxp3 in the expression of Lats2 in both normal and malignant breast epithelia**

Our gene array analysis showed that induction of FOXP3 in breast cancer cell line MCF7 leads to increased LATS2 expression (16). To determine whether endogenous Foxp3 regulates Lats2 in murine mammary epithelial cells, we used the Scurfy (sf) mice with a spontaneous mutation of the X-linked Foxp3 gene (a dinucleotide insertion, Foxp3sf) to determine the impact of Foxp3 inactivation on the expression of Lats2. Because the homozygous mutation caused lethal autoimmune diseases in the immune competent mice, we crossed the mutation into Rag2−/− mice that lack T and B cells. We isolated mammary epithelial cells from Rag2−/− Foxp3−/− and Rag2−/− Foxp3sf/ mice and prostate epithelial cells from Rag2−/− Foxp3−/− and Rag2−/− Foxp3sf/ mice and compared the level of Lats1 and Lats2 transcripts by real-time PCR. As shown in Figure 1A (top), epithelial preparation from the Foxp3sf/ mice showed a

![Figure 2. FOXP3 is necessary and sufficient for expression of LATS2 gene in normal and malignant human mammary epithelial cells. A, silencing FOXP3 in MCF10A cells reduces LATS2 expression. The levels of FOXP3 (top) and LATS2 (bottom) in 2 scrambled or 2 FOXP3 siRNA-transfected cells as determined by real-time RT-PCR. Data shown are mean ± SD of 3 independent experiments. B, induction of LATS2 mRNA by FOXP3 in MCF7 Tet-off system. MCF7 cells with Tet-off expression of either GFP control or GFP in conjunction with FOXP3 were cultured for 48 or 72 hours in the absence of doxycyclin. The LATS2 mRNA was quantified by real-time PCR. The induction of FOXP3 mRNA is shown at the bottom. Data shown are mean ± SD of 3 independent experiments.](cancerres.aacrjournals.org)
significant reduction in both Lats1 and Lats2 mRNA. Similar reductions were observed in the mutant prostate tissue (Fig. 1A, bottom). Because the Scurfy mutation cause frameshift and nonsense-mediated decay of mRNA, a significant reduction of Foxp3 transcript was also observed in the Scurfy mice. Furthermore, because the level of Lats1 mRNA was 10-fold less than that of Lats2, we have focused on regulation of Lats2 transcription by Foxp3.

When the lysates were compared for Lats2 and Yap proteins, it is clear that the Lats2 protein level was substantially reduced in the Foxp3-deficient epithelial cells from mammary and prostate glands (Fig. 1B). Correspondingly, a selective reduction in phosphorylated Yap was observed (Fig. 1B). We also carried out immunohistochemical analysis of both benign and cancerous tissue from the Rag2−/− and Rag2−/− Foxp3−/+ mice. As shown in Figure 1C, Lats2 protein was barely detectable in the epithelial cells from the Foxp3−/+ mice and completely absent from Foxp3−/− tumors. Consistent with tumor suppressor activity, the spontaneous mammary tumors were observed in the Rag2−/− Foxp3−/+ but not in the Rag2−/− Foxp3−/− mice (data not shown). Consistent with a role for Foxp3 in Lats2 expression, Foxp3 knockdown in murine mammary tumor cell line TSA (18) leads to reduction of lats2 transcripts and protein (Fig. 1D).

To study the role for FOXP3 in LATS2 expression in human cells, we tested the effect of FOXP3 silencing on immortalized human epithelial cell line MCF10A (19). As shown in Figure 2A, shRNA silencing of FOXP3 caused a major reduction of LATS2
transcript (Fig. 2A). As a complementary approach, we used a Tet-off system to test the effect of inducible expression of FOXP3 on LAT2 transcripts in the human breast cancer cell line MCF7. As shown in Figure 2B, induction of FOXP3 progressively increased LAT2 transcripts. The increase reached more than 10-fold at day 3 in the MCF-7 cells.

To determine whether FOXP3 contribute to LAT2 expression in the primary tumor samples, we used immunohistochemistry to test whether LAT2 expression correlates with that of nuclear FOXP3. As shown in Supplementary Table S2, 71% of nuclear FOXP3+ tumor samples expressed detectable LAT2, whereas only 46% of FOXP3+ samples expressed detectable LAT2. Chi-square analysis indicated a statistically significant correlation between LAT2 and nuclear FOXP3 expression.

**FOXP3 binding to the LAT2 promoter is essential for LAT2 transcription**

We searched the 5′ sequence of the LAT2 gene for FOXP3 recognition motifs (forkhead binding motif 5′-RYMAAYA or 5′-YRKTTTR; R = A, G; Y = C, T; M = A, C; K = G, T). The potential binding motifs in LAT2 promoter were diagramed in Figure 3A (top). We used real-time PCR to determine the amounts of specific DNA sequence in chromatin immunoprecipitates of anti-FOXP3 mAb. The MCF7 tumor cells with induced FOXP3 expression were used as source of chromatin. As shown in Figure 3A, of the 8 regions analyzed, all but one (B3) showed specific binding to FOXP3. To identify a functional element for FOXP3-mediated regulation of LAT2, we generated luciferase reporters consisting of 4 overlapping DNA fragments that cover all of the forkhead binding motifs and tested the effect of FOXP3 on the reporters. As shown in Figure 3B, FOXP3 cDNA strongly stimulated all 4 regions of the LAT2 promoter. Although the stimulation of P4 seemed less robust in this experiment, multiple experiments did not support the reduced promoter activity of this region. Therefore, P4 likely contained all necessary FOXP3 responsive cis element. To identify the FOXP3 responsive element in the P4, we deleted 3 FOXP3 binding sites in the region, one at a time, and tested their response to FOXP3 cDNA. As shown in Figure 3C, whereas mutation of B6 and B7 had no effect on response to FOXP3, that of B8 eliminated FOXP3 response of the LAT2 promoter. Therefore, B8 is the essential FOXP3 response element in the LAT2 promoter.

**FOXP3 activation of the Hippo pathway in normal mouse and human mammary epithelial cells**

YAP is the effector molecule repressed by the Hippo pathway and is a coactivator in gene transcription. Although LATS-phosphorylated YAP will be degraded (7), unphosphorylated YAP will translocate into nuclear to form complex with TEAD1-4 to activate gene transcription (20). To test regulation of YAP phosphorylation by FOXP3, we transfected wild-type (WT) or mutant FOXP3 cDNA in conjunction with YAP into 293T cells. Two days after transfection, YAP phosphorylation was determined by immunoblot. As shown in Figure 4A, FOXP3 increased YAP phosphorylation as revealed by antibody specific for phosphor-S127 and by electrophoresis mobility (Figure 4A, right). Mutations that either prevent FOXP3 dimerization (delta252E; ref. 21) or DNA binding (A341F342; ref. 22) abrogated this effect. These data suggest that FOXP3 regulates YAP phosphorylation through its role in gene regulation. Further, the impact of FOXP3 is achieved through LATS as the LAT2 kinase dead mutant (LAT2-K/R), which was known as a dominant negative inhibitor of LATS (7), abrogated YAP phosphorylation. We used a reporter system consisting of a 5x UAS-luciferase reporter and a Gal4 DNA binding domain fused to

![Figure 4](https://example.com/figure4.png)

**Figure 4.** FOXP3 regulates phosphorylation (A) and transcriptional coactivator function of YAP (B): requirement for DNA binding and dimerization of FOXP3 and endogenous LAT2. A, mutational analyses suggest the requirement for FOXP3-mediated transcription of LAT2 in FOXP3-induced YAP phosphorylation. Left, the impact of FOXP3 mutations; right, the effect of dominant negative LAT2. 293T cells were transfected with either WT or mutant FOXP3 cDNA in conjunction with either vector alone or dominant negative mutant of LAT2. At 48 hours after transfection, the transfectants were starved for 8 hours and then lysed for Western blots with antibodies specific for YAP, p-YAP, Myc-tag, or V5-tag. B, FOXP3 inhibited YAP activity. TEAD4 luciferase reporter assay was used to measure coactivation by TEAD4 and YAP. WT or mutant FOXP3 were transfected in conjunction with YAP and Gal4-TEAD4 and 5x UAS-luciferase reporter into 293 T cells. TEAD4 luciferase activity was measured and normalized to renila activity. Data shown are mean ± SD from 3 independent experiments.
Tea4(Gal4-Tea4) to test the effect of FOXP3 on coactivator activity of YAP. As showed in Figure 4B, without YAP cDNA, Gal4-Tea4 showed very low basal activity. With YAP coexpression, Gal4-Tea4 reporter was strongly activated. Transfection of FOXP3 reduced reporter activity. Again, the inhibition of the YAP activity is likely mediated by LATS as the LATS2 kinase dead mutant (LATS2-K/R) abrogated FOXP3 function.

CyclinE and Diaph2 are two well known YAP targets (20). To determine whether Foxp3 regulates YAP function in vivo, we isolated mammary epithelial cells from Rag2−/− Foxp3+/+ and Rag2−/− Foxp3−/− mice by real-time PCR. A and B, right, expression of CYCLINE1 and DIAPH2 mRNA in MCF10A cells transfected with either scrambled or FOXP3 shRNA. Transfectants were selected by puromycin for 2 weeks. Stable clones were cultured for RNA analysis. Data shown in A and B are mean ± SD from 3 independent experiments. C, impact of FOXP3 silencing on acinar formation in MCF10A. MCF10A cell lines transduced with lentiviral vectors control or shRNA were cultured in matrigel medium. Low-power (×10) images of acinar sizes (4',6-diamidino-2-phenylindole, DAPI) and expression of FOXP3 (green) and LATS2. High-power (×60) images of acinar structure and FOXP3 and LATS2 expression were shown in right corner of each picture. D, measurement of acinar size. The sizes were shown by relative area, measured at 14 days after culture. Data shown are mean ± SD of relative sizes. The mean sizes of the scrambled samples were artificially defined as 1.0.

FOXP3 Regulates LATS2 interaction contributes to tumor suppression

An important issue is whether Foxp3-mediated Lats2 expression contributes to tumor suppressor function of Foxp3. To address this issue, we tested whether the dominant-negative mutant of Lats2 abrogated tumor suppressor activity. As shown in Figure 6A, Foxp3 significantly inhibited the growth of murine mammary tumor cell lines TSA in vitro, as we had previously reported. This inhibition seemed to require Foxp3 dimerization and DNA binding, as the inactive Foxp3 mutants failed to suppress growth of TSA in vitro. Importantly, the...
kinase dead mutant of the Lats2 substantially diminished tumor suppressor function of Foxp3.

The growth inhibition pattern was largely recapitulated when the tumor cells were inoculated into the mammary fat pad. A typical example is presented in Figure 6B and the growth kinetics of TSA transfected with vector alone, Foxp3 or Foxp3 + Lats2-K/R mutant is shown in Figure 6C. These data show that growth inhibitory function of FOXP3 is attenuated by Lats2-K/R. To test whether FOXP3 inhibits cell growth in prostate cancer cells, a prostate cancer cell line LNCAP was transfected with FOXP3 alone or FOXP3 plus LATS2 kinase dead mutant LATS2-K/R. The results showed that LATS2-K/R mutant reversed the inhibition of FOXP3 on LNCAP cell growth, thus implying LATS2 as a downstream target for FOXP3-mediated growth inhibition.

**FOXP3 defects contribute to Hippo inactivation in human prostate cancer**

We have recently reported overexpression of YAP protein (7) and downregulation of nuclear FOXP3 proteins (13) in prostate cancer samples. To determine whether FOXP3 downregulation contributes to defective Hippo pathway in prostate cancer, we analyzed expression of LATS2, YAP, and FOXP3 mRNA in microdissected samples. Much like FOXP3 transcripts, LATS2 is downregulated in overwhelming majority of microdissected cancer cells, in comparison with normal prostate epithelial from the same patients (Fig. 7A, top). Moreover, we have recently uncovered 4 of 20 samples that harbor somatic FOXP3 missense mutations (13). As shown in Figure 7A (bottom), in each of the 4 cases, the tumor samples show greatly reduced LATS2 levels. Interestingly, downregulation of LATS2 strongly correlates with that of FOXP3 (Fig. 7B, top). In contrast, the levels of YAP transcripts show no correlation to that of FOXP3 ($r^2 < 0.5$; Fig. 7B, bottom). Therefore, FOXP3 defects are likely a major determinant of LATS2 levels in prostate cancer. Because either overexpression of YAP or downregulation of LATS2 can lead to activation of the hippo pathway, we plotted the mRNA levels of YAP and Lats of the 20 microdissected tumor samples. As shown in Figure 7C, the two genes seemed to be independently regulated.

A major indication of Hippo activation is accumulation of YAP protein in the nuclei. Although the cohort is too small to compare the relative importance of the two events, we were interested in whether downregulation of LATS2 could lead to nuclear accumulation of YAP in samples that showed no or little upregulation of YAP mRNA. We tested 3 cases of prostate cancer samples in which YAP expression is not significantly
elevated (red, green, and purple dots in Fig. 7C). In all 3 cases, clear accumulation of nuclear YAP was observed (see Fig. 7D for an example).

It is possible that the increase of nuclear YAP is caused by mutation of the LATS2 phosphorylation sites (S127 and S347). To address this possibility, we carried out sequence analysis of exons 2 and 6 (which encodes part of YAP that contains S127 and S347, respectively) of the YAP gene in microdissected samples. Our data revealed that none of the 20 cancer samples tested had mutation in the 2 exons. Because FOXP3 defects correlated with decreased LATS2 expression, and because LATS2 downregulation correlated with increased YAP protein in the absence of either activating YAP mutation or overexpression, it is likely that FOXP3 defects is a cause of YAP activation in the prostate cancer.

Discussion

FOXP3 is an X-linked tumor suppressor gene for breast and prostate cancers (13, 14, 23). As a transcription factor, FOXP3 has been shown to both downregulate oncogenes, including ERBB2 (14), SKP2 (15), and cMYC (13) and upregulate tumor suppressors such as p21 (16). Here we showed a tumor suppressor relay between the FOXP3 and Hippo pathways.

Our data show that FOXP3 directly interacts with the LATS2 promoter region to enhance the expression of LATS2 gene. The induction of LATS2 caused increased phosphorylation and reduction of total levels of oncoprotein YAP. Using a dominantly-negative LATS2 mutant, we showed a critical role for the FOXP3–LATS2 regulation in tumor suppressor function of FOXP3. The cross-regulation has been shown in both normal and malignant cells in mouse and human. These data not only

Figure 7. FOXP3 defects contribute to LATS2 mRNA downregulation and increased YAP protein level in human prostate cancer samples. A, downregulation of LATS2 mRNA in prostate cancer tissues in comparison with normal tissues from the same patients. The samples were isolated by laser-guided microdissections. Data from samples with no somatic missense mutation of FOXP3 were shown in the top, whereas those with missense mutations are shown in the bottom. P values were calculated by Wilcox 2-sample tests. B, correlations between downregulations of FOXP3 and LATS2 mRNA (top) or between FOXP3 and YAP mRNA (bottom). Only those with no missense mutation of the FOXP3 gene are presented. Data shown are ratios of transcript levels of cancer samples over benign tissues from the same patients. P values by Mann–Whitney U test. C, independent regulation of YAP and LATS2 mRNA in prostate cancer tissue. The ratio of the mRNA levels in microdissected cancer over benign epithelial tissue from prostate cancer patients was presented; quadroons show boundary of 2.5-fold increases of YAP and 2.5-fold decreases of LATS2 transcripts. The red, green, and purple dots indicate samples that were confirmed to have accumulation of nuclear YAP proteins. D, accumulation of nuclear YAP in prostate cancer samples with no increase in YAP transcripts. The data shown are representative of 3 samples in the group; the relative level of LATS2 and YAP mRNA in this sample is shown in C (purple dot).
further elucidate the mechanism of FOXP3-mediated tumor suppression but also broaden the impact of FOXP3 in pathogenesis of both breast and prostate cancer.

The FOXP3–LATS2 connection may contribute to the frequent loss of LATS2 transcripts and protein in breast (9) and prostate (10) cancers as high frequency of cancer sampled show mutation, deletion, and/or abnormal expression of FOXP3 (13–15, 24). The strong correlation between downregulation (or mutations) of FOXP3 and LATS2 expression, as shown here with microdissected prostate cancer samples, indicate that, at least for prostate cancer, defects in FOXP3 are likely a major mechanism for LATS2 loss. Our data from breast cancer TMA samples also show a significant correlation between loss of nuclear FOXP3 protein and LATS2 protein in cancer cells. However, the correlation is less striking than the prostate cancer samples. This variance in degree of correlations in the 2 cancers may be caused by the higher sensitivity and accuracy of real-time PCR analysis of microdissected samples than immunohistochemistry of TMA samples. Alternatively, it is also possible that the relative importance of FOXP3 defects in LATS2 downregulation differs in the 2 cancer types.

It is well documented that YAP protein is frequently upregulated in human cancer, including prostate (7), breast (25), colon (25), ovary (25), lung (25, 26), and liver (7, 27) cancers. At least 3 mechanisms can be involved in the elevation of the YAP protein. First, YAP gene resides in 11q22, which is amplified in colon (25), ovary (25), lung (25, 26), and liver (7, 27) cancers. This amplification of the YAP gene of 20 prostate cancer samples revealed no somatic mutation in the exon encoding the phosphorylation sites that target it for degradation could contribute to YAP upregulation. Thirdly, mutation of YAP regulatory region may be involved in YAP upregulation as LATS2-mediated phosphorylation targets YAP for degradation (6, 7). Because LATS2 downregulation strongly associates with FOXP3 downregulation in prostate and breast cancers, FOXP3 defects may contribute to YAP upregulation. Thirdly, mutation of YAP phosphorylation sites that target it for degradation could result in YAP accumulation. However, our sequencing of YAP gene of 20 prostate cancer samples revealed no somatic mutation in the exon encoding the phosphorylation sites. Therefore, YAP mutation is unlikely a major cause of YAP protein upregulation in prostate cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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