Sustained Adrenergic Activation of YAP1 Induces Anoikis Resistance in Cervical Cancer Cells

Yang Li, Shanshan Yang, Nouara C. Sadaoui, ..., Steve W. Cole, Susan K. Lutgendorf, Anil K. Sood

asood@mdanderson.org

HIGHLIGHTS

Daily restraint stress increases tumor growth and metastatic tumor burden

Norepinephrine protects cervical cancer cells from anoikis

Norepinephrine induces YAP1 dephosphorylation and nuclear translocation

Norepinephrine-induced anoikis resistance can be reversed by propranolol

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to establish such a model. First, to identify cell lines that could be affected by stress hormones, we tested Given the lack of established models of cervical cancer for assessing the effects of chronic stress, we sought Chronic Stress Promotes Cervical Cancer Growth 

RESULTS

A substantial number of studies have established an important role for biobehavioral stress factors in cancer progression (Antoni et al., 2006). Previously, we demonstrated that stress hormones affect tumor pathogenesis at multiple levels, our understanding of the underlying mechanisms is in its infancy and needs to be expanded. In this study, we established a preclinical orthotopic mouse model of cervical cancer to determine the physiologic effects of chronic stress in vivo and identified YAP1 activation as a potential stress effector involved in anoikis resistance, which promotes cervical cancer progression.

INTRODUCTION

Cervical cancer is the fourth most common malignancy in women worldwide, with an estimated 570,000 new cases in 2018, representing 6.6% of all cancers in women (Bray et al., 2018). Human papillomavirus (HPV) infection is a well-recognized causative factor for cervical cancer (Burd, 2003). It has been reported that severely stressful life events were associated with a 62% increased risk of HPV16 infection, high viral load, and recurrent infection (Lu et al., 2016); psychologic distress had an increased risk of cervical cancer-specific mortality (Lu et al., 2019). Chronic stress is thought to suppress protective immunity that is critical for eliminating immunogenic cancers (e.g., squamous cell and basal cell carcinomas) and virally associated cancers (e.g., HPV-associated cervical, anal, and oral cancers) (Antoni and Dhabhar, 2019). At present, the molecular pathways by which chronic stress affects cervical cancer progression are not well understood.

In this study, we established a preclinical orthotopic mouse model of cervical cancer to determine the physiologic effects of chronic stress in vivo and identified YAP1 activation as a potential stress effector involved in anoikis resistance, which promotes cervical cancer progression.

SUMMARY

Chronic stress-related hormones modulate tumor pathogenesis at multiple levels; however, the molecular pathways involved in stress and cervical cancer progression are not well understood. We established a preclinical orthotopic mouse model of cervical cancer and used the model to show that daily restraint stress increased tumor growth and metastatic tumor burden. Exposure to norepinephrine significantly protected cervical cancer cells from anoikis. We demonstrated that YAP1 was dephosphorylated and translocated from the cytoplasm to the nucleus by norepinephrine, a process initiated by ADRB2/cAMP/protein kinase A activation. Furthermore, anoikis resistance and YAP1 activation induced by norepinephrine could be rescued by a broad β-adrenergic receptor antagonist, propranolol. Collectively, our results provide a pivotal molecular pathway for disrupting pro-tumor neuroendocrine signaling in cervical cancer.

RESULTS

Chronic Stress Promotes Cervical Cancer Growth In Vivo

Given the lack of established models of cervical cancer for assessing the effects of chronic stress, we sought to establish such a model. First, to identify cell lines that could be affected by stress hormones, we tested...
multiple cervical (SiHa, CaSki, ME-180, C33A) and ovarian (SKOV3 and A2780) cancer cell lines for β-adrenergic receptors. Most of these cell lines expressed varying levels of β-adrenergic receptors, but the C33A and A2780 cell lines lacked β2-adrenergic receptor (ADRB2) expression (Figure S1).

To evaluate the potential role of chronic stress on cervical cancer in vivo, a restraint-stress orthotopic model of cervical cancer was established and characterized. Cervical cancer cells were injected directly into the cervix at the cervical-uterine junction. We found that SiHa and ME-180, but not CaSki cells, had tumorigenic potential. Three days later, nude mice were placed in a movement-restricted space for 2 h daily to mimic chronic stress (Thaker et al., 2006). The mice were subjected to daily restraint stress for 3 weeks, then euthanized 1 week after stress was ceased. Tumor growth was monitored weekly by IVIS bioluminescence imaging for the duration of the experiment. Bioluminescence imaging showed tumor localization at the cervix within the pelvic region (Figure 1A). Further necropsy showed lymph-vascular invasion of tumors and spread into the parametrium and pelvic wall (Figure 1B). Representative hematoxylin and eosin (H&E) staining of the tumor tissues is shown in Figure S2. This model resembles the disease observed in the clinic; thus, it was adopted by our laboratory for further studies. Animals subjected to daily restraint stress had significantly greater tumor weight (p < 0.05) and higher metastatic tumor burden (p < 0.05) than non-stressed control mice that received the same injection of SiHa cells (Figures 1C and 1D). Of note, the broad β-antagonist propranolol inhibited stress-induced increases in both tumor weight (p < 0.01) and metastatic burden (p < 0.01).

NE Induces Anoikis Resistance Mediated by Decreased YAP1 Phosphorylation and NF2

To identify potential mechanisms of NE action during cervical cancer progression, we performed reverse phase protein array (RPPA) analysis in SiHa cells treated with or without 10 μM NE for 12 h. As shown in the heatmap (Figure 1E) and volcano plot (Figure 1F), there were 26 upregulated and 26 downregulated proteins in the NE-treated group.

Then, we performed Gene Ontology (GO) analysis for functional enrichment. Among the most prominent GO functions in the differentially expressed genes in the NE treatment group, regulation of apoptosis process was significantly enriched (Figure 1G). Apoptosis can be induced by numerous triggers, including the loss of cell anchorage, or anoikis. Resistance to anoikis is a hallmark of metastasis, allowing tumor cells longer survival in the absence of matrix attachment and facilitating migration, reattachment, and colonization of secondary sites (Sood and Lutgendorf, 2011).

To validate whether NE could induce anoikis resistance, we cultured CaSki, ME-180, and C33A cervical cancer cells in ultra-low attachment plates, which allow for anchorage-independent growth. When treated with 10 μM NE for 72 h, CaSki (p < 0.05) and ME-180 (p < 0.0001) cells, which are ADRB2 positive, were significantly protected from anoikis, while the ADRB-negative C33A cells were not (p > 0.05) (Figure 2A). Similarly, SiHa, CaSki, and ME-180 cells had significantly greater migration and invasion when treated with NE, but C33A cells did not (Figure S3). Furthermore, immunohistochemical analysis of apoptosis in tumor sections revealed that cleaved caspase-3-positive cells in mice subjected to 21 days of daily restraint stress as described above were reduced compared with those among controls (p < 0.01; Figures 2B and 2C). These effects were attenuated in the group that was both stressed and treated with propranolol (p < 0.01; Figures 2B and 2C).

To uncover potential pathways involved in NE-induced anoikis in cervical cancer, additional computational analysis of differentially expressed genes using the Kyoto Encyclopedia of Genes and Genomes (KEGG) showed the top ten enriched pathways (Figure 2D): seven pathways in signal transduction, two in human
Figure 2. Norepinephrine (NE) Induces Anoikis Resistance and Putative Pathways in Cervical Cancer Cells

(A) Number of dead (SYTOX Red-positive, black) and living (SYTOX Red-negative, white) CaSki, ME-180, and C33A cells after 72 h of low attachment and/or co-incubation with 10 μM NE.

(B and C) (B) Representative immunohistochemistry and (C) quantification staining of apoptosis protein marker in mouse cervical tumors in various conditions. n = 6 mice per group. Scale bar is 200 μm.

(D) Kyoto Encyclopedia of Genes and Genomes (http://www.genome.ad.jp/kegg/) enriched putative pathways for 52 differentially expressed proteins by RPPA. Data are expressed as number of cleaved caspase-3-positive cells per high-power field. Experiments were repeated in triplicate. Data are presented as mean ± standard deviation (SD). Differences between treatment groups were determined by orthogonal contrasts and denoted as follows: *p < 0.05, **p < 0.01, ***p < 0.001; ns, no significance.
diseases (EGFR tyrosine kinase inhibitor resistance and endocrine resistance), and one in cellular processes (P53 signaling pathway). Among these, the Hippo signaling pathway, which has been reported to contribute to anoikis evasion (Zhao et al., 2012), has been identified as a candidate for NE-induced signaling. Its well-known downstream effector YAP1 (Howe and Juliano, 2000; Misra and Irvine, 2018) and upstream regulator NF2 (Reginensi et al., 2016) were both indicated in the enriched KEGG network (Figure 2D).

Our reverse phase protein array data indicated that phosphorylation level of YAP1S127 and total NF2 protein were significantly decreased by 82.60% and 90.42%, respectively, in NE-treated SiHa cells (Figures 1E and 1F). Consistent with this finding, when CaSki and ME-180 cells were exposed to 10 µM NE under both normal and low-attachment conditions, phosphorylated YAP1S127 and NF2 were significantly downregulated in CaSki and ME-180 cells (p < 0.01; Figures 3A and 3B) but not in C33A cells (ADRB2-negative; p > 0.05; Figure 3C).

YAP1 Activation Is Required for NE-Induced Anoikis Resistance
Phosphorylation of YAP1S127 generates a 14-3-3-binding motif responsible for YAP1 cytoplasmic retention (Zhao et al., 2007); if dephosphorylated, activated YAP1 can translocate into the nucleus and promote transcription of genes that in turn inhibit apoptosis (Kapoor et al., 2014; Yu and Guan, 2013; Yu et al., 2012a). We used immunofluorescence to visualize the intracellular localization of YAP1 in CaSki and ME-180 cells treated with or without 10 µM NE for 2 h and found a clear shift in YAP1 expression from the cytoplasm to the nucleus after NE treatment (Figures 4A and 4B). Consistently, nuclear YAP1 protein expression was significantly higher in mice exposed to restraint stress than those without restraint stress, and propranolol treatment completely abrogated this effect (Figures 4C and 4D). To determine whether similar findings are noted in human samples, we obtained cervical cancer samples from eleven patients. Levels of depressive scores were measured during the pre-surgical clinic visit 1 to 7 days prior to tumor resection. Based on the established threshold of CESD ≥16, six participants were determined to have high levels of biobehavioral risk factors, whereas five were low risk. Nuclear staining of YAP1 was significantly higher in patients with cervical cancer with high CESD scores than those without (p < 0.05; Figures 4E and 4F).

Next, we used two siRNAs to knock down YAP1 gene expression in CaSki cells (p < 0.01 and p < 0.05; respectively; Figure 4G) and ME-180 cells (p < 0.05 and p < 0.01, respectively; Figure 4H). NE 10 µM for 72 h inhibited anoikis in cervical cancer cells transfected with control siRNAs, whereas YAP1 siRNAs abrogated this NE-induced anoikis resistance in both CaSki (Figure 4I) and ME-180 (Figure 4J) cells under anchorage-independent conditions. In addition, when expression of NF2 was downregulated by two siRNAs (Figures S4A and S4B), phosphorylated YAP1S127 was decreased (Figures S4C and S4D), and NE-induced anoikis resistance was completely reversed (p < 0.05; Figures S4E and S4F).

NE Regulates Hippo-YAP1 Pathway via ADRB2-Mediated Signaling
We next delineated the signaling pathway involved in NE-mediated Hippo-YAP1 activation. Following NE treatment for 1–3 h, in addition to expression changes in NF2 and pYAP1S127, two core kinases in the Hippo pathway, mammalian Ste20-like kinase 1 (MST1) and large tumor suppressor kinase 1 (LATS1), were both dephosphorylated and inactivated (Figure 5A). When cells were pretreated with 10 µM propranolol, 10 µM atenolol (ADRB1 antagonist), or 10 µM ICI-118,551 (ADRB2 antagonist) for 1 h before NE exposure, NE-induced anoikis resistance was abrogated completely by propranolol and ICI-118,551 (Figures 5B and 5C). Furthermore, treatment with ICI-118,551 or propranolol resulted in abrogation of the NE-mediated decrease in YAP1 phosphorylation (Figure 5D); ADRB2 silencing by siRNAs in CaSki and ME-180 cells (Figures S5A and S5B) resulted in abrogation of NE-mediated YAP1 dephosphorylation (Figures S5C–S5E). When ADRB2 was overexpressed using the p6596 ADRB2 plasmid in C33A cells, YAP1 was dephosphorylated by NE (Figure S6).

Because cAMP is an important component of the ADRB2 signaling pathway, we examined intracellular cAMP levels after NE treatment. Relative to controls, treatment with 10 µM NE for 30 min increased cAMP levels by 2.92-fold in CaSki (p < 0.05), 3.32-fold in SiHa (p < 0.05), and 3.97-fold in ME-180 (p < 0.05) cells, but no change was noted in C33A cells (p > 0.05) (ADRB2-negative; Figure 6A). Treatment with 10 µM forskolin (cAMP activator) induced similar anoikis resistance in CaSki (p < 0.05) and ME-180 (p < 0.05) cells (Figure 6B). Exposure to 10 µM forskolin for various durations decreased pYAP1S127 expression, much like NE treatment did (Figure 6C). Protein kinase A (PKA) is an important protein downstream of
Figure 3. NE Decreases YAP1 Phosphorylation and NF2 Expression in CaSki and ME-180 Cells

Western blot analysis of phosphorylated YAP1S127, total YAP1, and NF2 in CaSki (A), ME-180 (B), and C33A (C) cultured in normal plates (NP) or ultra-low attachment plates (LP) with or without 10 μM NE co-incubation. β-Actin was used as a loading control. The immunoblots are on the left, and quantifications of band intensity relative to β-actin are on the right (n = 3, data represent the mean ± SD). Differences between treatment groups were determined by orthogonal contrasts and denoted as follows: *p < 0.05, **p < 0.01; ns, no significance.
cAMP, and inhibition of PKA (using 10 μM H-89) markedly protected against NE-induced anoikis resistance (Figures 6D and 6E).

**DISCUSSION**

The key finding of this study is that chronic stress protects cervical cancer cells from anoikis and this protection promotes malignant tumor progression. Here, we developed a preclinical orthotopic mouse model of cervical cancer to provide a new understanding of the effects of chronic stress on cervical cancer growth and metastasis. HPV-16 is the most common HPV genotype in cervical cancer; ME-180 cells are HPV-68 positive; the oncogenic ability is lower than HPV-16, so ME-180 was not selected for orthotopic model. Even though Caski cell and SiHa cells both are HPV-16 positive, we found that Caski did not have orthotopic tumorigenic potential in the mouse cervix, partially attributed to the fact that Caski cells are derived from a metastatic site (small intestine). Thus, SiHa cells (HPV16 positive) were used for the orthotopic mouse model. Our data obtained using this model indicate that the neuroendocrine stress response by NE directly induces anoikis resistance through a signaling pathway mediated by Hippo-YAP1 that is initiated by ADRB2/cAMP/PKA activation. Anoikis is a process by which normal cells undergo apoptosis when detached from the surrounding extracellular matrix. Avoidance of anoikis is an essential prerequisite for tumor metastasis; it provides a selective advantage that allows metastatic cancer cells to transit to new sites for attachment (Frisch and Screaton, 2001). Previously, we demonstrated an important role for YAP1 signaling in blocking anoikis (Haemmerle et al., 2017). Similarly, other groups have demonstrated Hippo pathway-mediated anoikis inhibition in hepatocellular carcinoma (Cheng et al., 2018); YAP/TAZ activation by ASPP1 leads to anoikis resistance (Vigneron et al., 2010). In the present study, we implicate sustained adrenergic stimulation in inducing cervical cancer anoikis resistance by inhibition of the tumor suppressive Hippo-YAP1 pathway.

The Hippo-YAP1 pathway plays an important role in regulating cell proliferation, death, and differentiation (Yu and Guan, 2013). The tight control of this pathway and its cross talk with other signaling pathways is critical for tumorigenesis and cancer progression (Yu et al., 2013). Several modulators of the Hippo-YAP1/TAZ pathway have been identified via extensive genetic and biochemical analyses (Yu and Guan, 2013; Zhao et al., 2010, 2011); the Hippo-YAP1/TAZ pathway is robustly regulated by a wide range of signals and their corresponding G protein-coupled receptors (Yu et al., 2012b). Here, we found that ADRB2, a classic G protein-coupled receptor, mediates NE-induced YAP1 activation in cervical carcinogenesis, which indicates that neuroendocrine stress signaling takes part in non-canonical regulation of the Hippo-YAP1 pathway.

Activation of ADRB2 by epinephrine, NE, or specific agonists typically results in Gs-dependent activation of adenylate cyclase and a subsequent increase in intracellular cAMP (Tan et al., 2007). This increase in cAMP stimulates PKA to phosphorylate multiple target proteins, including transcription factors of the CREB/ATF and GATA families (Cole and Sood, 2012; Rockman et al., 2002). Similarly, we found that intracellular cAMP stimulation in cervical cancer cells were increased by NE stimulation and NE-induced effects could be mimicked by cAMP activation and blocked by a PKA antagonist. Neurofibromin 2 (NF2, also known as merlin), a tumor suppressor and an upstream component of the Hippo pathway, is a direct target of PKA (Alfthan et al., 2004). Our study identified a new functional role for NF2 as a key molecular effector that links adrenergic signaling to the downstream Hippo-YAP1 pathway and tumor progression.

Considering the role of stress-induced suppression of protective immune responses in infection-related cancer (Antoni and Dhabhar, 2019), HPV-driven cervical cancer is potentially much more sensitive to effects of chronic stress than other cancers. Lu et al. found that major life events, including bereavement, severe illness of a family member, divorce, and being between jobs, were prevalent among patients with cervical cancer (37.4%) (Lu et al., 2019). We previously reported that intra-tumoral NE levels in primary ovarian carcinomas are linked to both disease severity and patient psychosocial characteristics (Lutgendorf et al., 2009),
but very little data about NE levels and β-adrenergic signaling in cervical cancer have been reported to date. In our study, chronic stress induced YAP1 nuclear translocation and anoikis resistance in cervical cancer, and these effects were attenuated by propranolol. These data indicate that pharmacologic inhibition of adrenergic receptors may have therapeutic relevance in cervical cancer. Emerging clinical data also link the use of non-selective β-adrenergic receptor blockers with reduced cancer progression (Barron et al., 2011; De Giorgi et al., 2011; Melhem-Bertrandt et al., 2011). Of note, results of retrospective studies are prone to immortal time bias and need to be further validated in prospective studies (Weberpals et al., 2016).

In summary, our data represent a new understanding of YAP1 activation in response to sustained adrenergic signaling in cervical cancer models (Figure 7). Protective interventions targeting the neuroendocrine system may provide a biologically plausible method to prevent cervical cancer progression.

Limitations of the Study
Although our results demonstrate that norepinephrine induces anoikis resistance in cervical cancer, it is possible that other pathways (e.g., cortisol) could have similar or broader effects. To what extent these stress-related hormones are important in clinical context would require further work. Moreover, in the absence of available syngeneic mouse models, we used cross-species cell line-derived tumor xenograft (CDX) mouse models in this study to understand the role of stress in mediating cancer cell-driven mechanisms of cervical cancer progression. However, it is possible that the tumor microenvironment may also play an important role; immune-competent models would be required for such work.

Resource Availability
Lead Contact
Anil K. Sood, MD Department of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center, 1155 Herman Pressler, Houston, TX 77030, Tel 713-745-5266, Fax 713-792-7586 (Email: asood@mdanderson.org).

Materials Availability
No new materials were generated in this study.

Data and Code Availability
The raw data that support the findings of this study are available from the corresponding authors, upon request.

METHODS
All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101289.

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AUTHOR CONTRIBUTIONS
Conceptualization, Y.L., S.Y., and A.K.S.; Methodology, Y.L., N.C.S., L.W., Y.K., L.S.M., Y.S.; Investigation, Y.L., S.Y., S.Z., C.L. and K.L.; Writing – Original Draft, Y.L. and S.Y.; Writing – Review & Editing, Y.L., W.H., L.M.R., Y.L., S.W.C., S.K.L., S.K.D., and A.K.S.; Funding Acquisition, Y.L., S.Y., W.H., and A.K.S.; Supervision, A.K.S.

DECLARATION OF INTERESTS
A.K.S.: Consulting (Merck, Kiyatec), shareholder (BioPath), research funding (M-Trap).

Figure 6. cAMP and Protein Kinase A (PKA) Mediate NE-Induced Anoikis Resistance and YAP1 Activation
(A) Baseline and NE-induced levels of intracellular cyclic adenosine monophosphate (cAMP) in cervical cancer cells measured by enzyme-linked immunosorbent assay (n = 3).
(B) Effects of 10 μM forskolin (cAMP activator) on anoikis in CaSki and ME-180 cells.
(C–E) (C) Expression pattern of pYAP1S127 and total YAP1 at different time points on western blot analysis (n = 2). The immunoblot is at the top, and quantification of band intensity relative to β-actin is below. Effect of H-89 (PKA antagonist, 10 μM or 1 μM incubated for 1 h before NE exposure) on anoikis in CaSki (D) and ME-180 (E) cells.
*p < 0.05; **p < 0.01; ***p < 0.001; ns, no significance.

Figure 7. Working Model of NE-Induced YAP1 Activation and Anoikis Resistance in Cervical Cancer Cells
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Supplemental Information

Sustained Adrenergic Activation of YAP1
Induces Anoikis Resistance
in Cervical Cancer Cells

Yang Li, Shanshan Yang, Nouara C. Sadaoui, Wei Hu, Santosh K. Dasari, Lingegowda S. Mangala, Yunjie Sun, Shuangtao Zhao, Linghua Wang, Yuan Liu, Lois M. Ramondetta, Ke Li, Chong Lu, Yu Kang, Steve W. Cole, Susan K. Lutgendorf, and Anil K. Sood
**Supplemental Figures**

**Figure S1.** Expression of β-adrenergic receptors ADRB1, ADRB2, and ADRB3 in four cervical and two ovarian cancer cell lines. Red box: Absence of ADRB2 expression. Figure S1. Related to Figure 2.

**Figure S2.** Representative H&E staining of cervical cancer tissues from mice (×100 magnification). Samples were obtained from: (A) control mice without restraint stress; (B) mice exposed to daily restraint stress; (C) mice with propranolol, no restraint stress; (D) mice with propranolol and daily restraint stress. Scale bar is 200 μm. Figure S2. Related to Figure 1.
Figure S3. Migration and invasion assay of SiHa, CaSki, ME-180 and C33A cells treated with or without 10 µM NE. Cells per HPF (200×) were counted. Error bars, SD. *P<0.05; **P<0.01; ns = no significance. Figure S3. Related to Figure 2.
Figure S4. NE-induced anoikis resistance and YAP1 activation are dependent on NF2. Quantitative reverse transcription–polymerase chain reaction (A, B) and Western blot analysis (C, D) in CaSki and ME-180 cells showing efficacy of NF2 knockdown at the RNA and protein levels using two siRNAs. Bar graphs showing number of dead (STYOX Red–positive, black) and living (SYTOX Red–negative, white) CaSki (E) and ME-180 (F) cells after 72 hours of low attachment. Bars and error bars represent mean ± SD. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; ns = no significance. CTRL: control. Figure S4. Related to Figure 4.
Figure S5. NF2 and YAP1 expression changes after ADRB2 knockdown in CaSki and ME-180 cells. Quantitative reverse transcription–polymerase chain reaction (A, B) in CaSki and ME-180 cells showing efficacy of ADRB2 knockdown at the mRNA level using two siRNAs. (C) Western blot analysis of YAP1 and NF2 expression in CaSki and ME-180 cells transfected with ADRB2 siRNAs. (D, E) Quantification of band intensity relative to β-actin is shown. Data represent the mean ± SD. **P<0.01, and ***P<0.001. CTRL: control. Figure S5. Related to Figure 5.
Figure S6. NF2 and YAP1 expression changes after ADRB2 overexpression in C33A cells. Quantitative reverse transcription–polymerase chain reaction (A) in C33A cells showing efficacy of ADRB2 overexpression at the mRNA levels with different amount of plasmid. (B) Western blot analysis of pYAP1, YAP1 and NF2 expression in C33A cells after transfection with ADRB2 plasmid (pADR2). (C) Quantification of band intensity relative to β-actin is shown. Data represent the mean ± SD. **P<0.01, and ***P<0.001. ns = no significance. Figure S6. Related to Figure 5.
Transparent Methods

All experiments were approved and confirmed to conform to the relevant regulatory standards of The University of Texas MD Anderson Cancer Center.

STAR Methods

KEY RESOURCES TABLE

| REAGENT or RESOURCE                  | SOURCE                     | IDENTIFIER       |
|--------------------------------------|----------------------------|------------------|
| Antibodies                           |                            |                  |
| Anti-YAP1                            | Cell Signaling Technology  | Cat#14074;       |
|                                      |                            | RRID:AB_2650491  |
| Anti-phospho-YAP (Ser127)            | Cell Signaling Technology  | Cat# 13008;      |
|                                      |                            | RRID:AB_2650553  |
| Anti-Phospho-LATS1 (Ser909)          | Cell Signaling Technology  | Cat# 9157;       |
|                                      |                            | RRID:AB_2133515  |
| Anti-LATS1                           | Cell Signaling Technology  | Cat# 3477;       |
|                                      |                            | RRID:AB_2133513  |
| Anti-MST1                            | Cell Signaling Technology  | Cat# 3682;       |
|                                      |                            | RRID:AB_2144632  |
| Anti-Phospho-MST1 (Thr183)/MST2 (Thr180) | Cell Signaling Technology | Cat# 49332,     |
|                                      |                            | RRID:AB_2799355  |
| Anti-NF2                             | Abcam                      | Cat# ab88957;    |
|                                      |                            | RRID:AB_2042307  |
| Anti-β-Actin                         | Sigma-Aldrich              | Cat# A5441;      |
|                                      |                            | RRID:AB_476744   |
| Anti-Cleaved Caspase-3               | Cell Signaling Technology  | Cat# 9661;       |
|                                      |                            | RRID:AB_2341188  |
| Alexa Fluor® 488 Goat AntiRabbit IgG | Jackson ImmunoResearch     | Cat# 111-546-047;|
|                                      |                            | RRID:AB_2338056  |
| ECL™ Anti-Mouse IgG, Horseradish Peroxidase | GE Healthcare         | Cat# NA931;     |
|                                      |                            | RRID:AB_772210   |
| ECL™ Anti-Rabbit IgG, Horseradish Peroxidase | GE Healthcare         | Cat# GENA934,    |
|                                      |                            | RRID:AB_2722659  |
| Chemicals, Peptides, and Recombinant Proteins |                  |                  |
| L-(-)-Norepinephrine (+)-bitartrate salt monohydrate | Sigma-Aldrich        | Cat# N5785      |
| Chemical Name                        | Supplier      | Catalog Number |
|-------------------------------------|---------------|----------------|
| (±)-Propranolol hydrochloride       | Sigma-Aldrich | Cat# P0884     |
| ICI 118,551 hydrochloride           | Sigma-Aldrich | Cat# L127      |
| Forskolin                           | Sigma-Aldrich | Cat# 344270    |
| InSolution H-89, Dihydrochloride    | Sigma-Aldrich | Cat# 371962    |
| Atenolol                            | Tocris        | Cat# 0387      |
| Halt Protease Inhibitor Cocktail (100X) | Thermo Fisher Scientific | Cat# 78438 |
| Tris base                           | Fisher Scientific | Cat# BP152-5 |
| Permount                            | Fisher Scientific | Cat# SP15-100 |
| Matrigel                            | BD Biosciences | Cat# 356231    |
| Lipofectamine RNAiMAX transfection reagent | Invitrogen | Cat# 13778500 |
| FuGENE® HD Transfection Reagent     | Promega       | Cat# E2311     |
| SYTOX® Red Dead Cell Stain          | Life technologies | Cat# S34859 |
| Power SYBR® Green PCR Master Mix    | Thermo Fisher Scientific | Cat# A25778 |
| Trizol                              | Invitrogen    | Cat# 15596-018 |

**Critical Commercial Assays**

| Assay Name                          | Supplier      | Catalog Number |
|-------------------------------------|---------------|----------------|
| Verso cDNA Synthesis Kit            | Thermo Fisher Scientific | Cat# AB1453B |
| cAMP Parameter Assay Kit            | R&B System    | Cat# KGE002B   |

**Experimental Models: Cell Lines**

| Cell Line   | Supplier | Catalog Number |
|-------------|----------|----------------|
| Human: SiHa | ATCC     | HTB-35         |
| Human: CaSki| ATCC     | CRM-CRL-1550   |
| Human: ME-180| ATCC   | HTB-33         |
| Human: C33A | ATCC     | HTB-31         |

**Oligonucleotides**

| Oligonucleotide | Supplier | Catalog Number |
|-----------------|----------|----------------|
| YAP1 siRNA1     | Sigma-Aldrich | SASI_Hs01_001824 03 |
### CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for all original resources and reagents presented in this manuscript should be directed and will be fulfilled by the Lead Contact, Anil K Sood (asood@mdanderson.org)

### Cell lines and tissue culture

Cervical cancer cell line CaSki was cultured with Roswell Park Memorial Institute (RPMI) 1640 medium, ME-180 was with McCoy's 5A medium, SiHa was with Minimum Essential Medium.
(MEM) and C33A was with Dulbecco's Modified Eagle Medium (DMEM); ovarian cancer cell line SKOV3 and A2780 were cultured with RPMI 1640 medium. All cell lines were purchased from the American Type Culture Collection and supplemented with 10% fetal bovine serum (FBS) and 0.1% gentamicin sulfate (Gemini Bio-Products) in 5% CO₂ at 37 °C. All experiments were performed at 70%-80% confluence, and cell lines were routinely screened for mycoplasma.

In vivo model of restraint-stress in an orthotopic model of cervical cancer

All in vivo experiments were approved by the Institutional Animal Care and Use Committee of The University of Texas MD Anderson Cancer Center. 8-12 weeks-old female athymic nude mice were obtained from Taconic Farms. Mice were randomized (10 mice per treatment) into four groups: 1) control with no stress, 2) control with restraint stress, 3) propranolol with no stress, and 4) propranolol with restraint stress. For restraint-stress experiments, nude mice were placed in a movement-restricted space for 2 hours daily as described previously (Thaker et al., 2006). On day 0, mice were underwent a mid-ventral laparotomy, during which they received an injection of 2×10^6 SiHa, CaSki or C33-A cancer cells in 100 μl of Hank's Balanced Salt Solution into the uterine cervix. From day 3, mice were subjected to this movement-restricted stress for 21 days. In the SiHa cervical cancer model, for 21 days, all mice received daily intraperitoneal injection of phosphate-buffered saline (PBS) or propranolol at 2 mg/kg. Seven days after stress was ceased, the mice were sacrificed by cervical dislocation and their tumors were dissected.

Patients and psychological measures

A total of 11 cervical cancer patients who had undergone primary surgical resection at Fudan University, Shanghai between November 2014 and September 2015 were included in this study. All the tumor samples were confirmed to be stage Ib1-IIa squamous cervical carcinomas. This study was approved by the Ethics Committee of the Obstetrics and Gynecology Hospital affiliated to Fudan University. Biobehavioral assessments were carried out using the Center for Epidemiologic Studies Depression Scale (CESD). The CESD is a validated 20-item scale that assesses depressive symptoms occurring in the prior week. Scores of higher than 16 indicate a high biobehavioral risk (Radloff, 1977; Weissman et al., 1977).

siRNA preparation and treatment
Lipofectamine RNAiMAX transfection reagent (Invitrogen) was used according to the manufacturer’s protocol to transiently transfect cells. Forward transfection with 40 nM siRNA was performed in cells in normal plates. Media was changed after 6 hours, and cells were collected for RNA or protein extraction at 48 hours after transfection. Cells in suspension, seeded in a low-attachment plate, were transfected with siRNAs (40 nM) by reverse transfection. Antibiotics were added 6 hours later, and NE was added 24 hours later. Cells were cultured for 72 hours for the anoikis assay.

**Plasmid transfection**

FuGENE HD Transfection Reagent (Promega) was used to transfect p6596 MSCV-IP-N-HA ADRB2 into C33A cells. Media was changed after 6 hours, and cells were collected for RNA or protein extraction at 72 hours after transfection.

**Anoikis assay in vitro**

Cells (5 × 10^5 per well) were seeded in an ultra-low attachment six-well plate (#3471, Corning) with incomplete media (no serum). Cells were pretreated with 10 μM PKA inhibitor (H-89), nonselective β-blocker (propranolol), selective ADRB1 antagonist (atenolol), or ADRB2 antagonist (ICI-118,551) for 1 hour and then treated with fresh prepared NE at 10 μM. For Western blot, cells were collected at 1-3 hours. For the anoikis assay, cells were treated with NE or blockers every 12 hours and collected at 72 hours. After incubation, the cells in suspension were harvested and centrifuged at 1500 RPM for 5 minutes. Pellets were washed with cold PBS supplemented with 0.02% FBS twice. Dead cells were quantified by flow cytometry using SYTOX Red Dead Cell Stain (Invitrogen) according to the manufacturer’s protocol and then analyzed by flow cytometry using a Beckman Coulter Gallios analyzer.

**RNA extraction and quantitative real-time PCR**

Total RNA was extracted using a Qiagen RNeasy kit and quantified by spectrophotometry (NanoDrop, Thermo Fisher Scientific). cDNAs were synthesized using the Verso cDNA kit (Thermo Fisher Scientific) according to the manufacturer’s instructions. Real-time polymerase chain reaction was performed using a 7500 Fast Real-Time PCR System (Applied Biosystems) with Power SYBR Green PCR Master Mix (Applied Biosystems). 18S was used as a housekeeping gene (RNA18S5, #HP220445, OriGene). The sequences of primers are presented in the Supplementary Table.
Western blotting analysis

Cell lysates were prepared using radioimmunoprecipitation assay buffer (50 mM Tris–HCl [pH 7.4], 150 mM NaCl, 1% Triton, 0.5% deoxycholate) supplemented with Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific). The protein concentrations were assessed using a BCA Protein Assay reagent kit (Pierce Biotechnology), and proteins were separated via sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Then, proteins were transferred onto a nitrocellulose membrane using electrophoresis (Bio-Rad Laboratories). All membranes were blocked with 5% nonfat dry milk in 1× Tris-buffered saline with Tween 20 for 1 hour and then incubated at 4 °C overnight with the primary antibodies: After washing with 1× Tris-buffered saline with Tween 20, membranes were then incubated with horseradish peroxidase–conjugated anti-mouse or anti-rabbit immunoglobulin G (1:2000; GE Healthcare Life Sciences) for 1 hour at room temperature and exposed using an enhanced ECL Western Blotting Substrate (Pierce Biotechnology). β-actin was used as loading control.

Reverse phase protein analysis and enrichment analysis

Protein lysates were isolated from cells as described above. Samples were diluted to 1.5 μg/μl in radioimmunoprecipitation assay buffer, denatured by 1% sodium dodecyl sulfate with β-mercaptoethanol and stored at −80 °C until further use. Reverse phase protein array was performed at the MD Anderson Functional Proteomics Reverse Phase Protein Array Core facility using 120 μg of protein per sample. All antibodies were validated previously (Tibes et al., 2006). To see associations between the identified gene modules and gene functions, we performed enrichment analyses using GO databases (Biological Process and Molecular Function, http://geneontology.org/) (Ashburner et al., 2000) and KEGG pathways (https://www.genome.jp/kegg/) (Kanehisa et al., 2004). The tools were used with the default options: a significance threshold of 0.05 for adjusted P value, at least two genes from the input list in the enriched category, and the whole genome as the reference background.

cAMP quantification

Intracellular levels of cAMP were quantified colorimetrically using a cAMP Parameter Assay Kit (#KGE002B, R&D Systems) according to the manufacturer’s instructions. Results are presented as pmol of cAMP per ml of protein.
**Immunofluorescence**

After culturing in the low attachment plate, the cells were collected and spun down on a coated glass coverslip (#08-774-383, Fisher Scientific), and then fixed in 4% paraformaldehyde for 20 minutes at room temperature, incubated in PBS/0.2% Triton X-100 for 10 minutes and blocked in PBS/10% FBS + 1% bovine serum albumin for 1 hour at room temperature. Thereafter, the fixed cells were incubated at 4 °C overnight with the primary anti-human YAP1 antibody. The next day, Alexa Fluor 488–labeled goat anti-rabbit secondary antibody (1:1000, #ab150077; Abcam) was added for 1 hour at room temperature. Hoechst 33342 (1:10,000, Molecular Probes) was used for nuclear counterstaining. Slides were mounted using ProLong Diamond Antifade Mountant (#P36965, Invitrogen).

**Immunohistochemical analysis**

Paraffin-embedded tissue slides were deparaffinized, dehydrated, and rehydrated and then processed in a series of xylene and alcohol washes, and antigen retrieval was performed in Diva Decloaker in a pressure cooker. Nonspecific epitopes were blocked in 4% fish gelatin for 1 hour, and the primary antibody, anti–cleaved caspase-3 or YAP1, was incubated overnight at 4 °C. Slides were incubated with horseradish peroxidase–conjugated secondary antibody (Jackson ImmunoResearch) at room temperature for 1.5 hours, and then 3,3′-diaminobenzidine (DAB, Invitrogen) was used to visualize the stains. Next, slides were counterstained with hematoxylin (Invitrogen).

**Migration and invasion assays**

24–well polycarbonate-membrane modified Boyden chambers (Corning) were used for both assays following previously described protocols (Armaiz-Pena et al., 2013). An 8-µm insert (#141006, Thermo Fisher Scientific) was used for CaSki cells, and a 12-µm Millicell cell insert (PIXP01250) was used for ME-180 and C33A cells. Single-cell suspensions were seeded into the upper wells at a concentration of 1×10^5 cells per well for the invasion assays and 7.5×10^4 cells per well for the migration assays. Cells were incubated at 37 °C in 5% CO₂ for 8-12 hours for migration and 24 hours for invasion. The membranes were fixed, stained, and counted by light microscopy at 200× for quantification.
**Statistical analysis**

Statistical analysis and data normality test were done using GraphPad Prism 8.0. Differences between two groups were evaluated using a two-tailed Student t-test or one-way analysis of variance. Results are presented as the mean ± standard error of the mean. For all statistical analyses, P<0.05 was considered statistically significant.

**Supplemental References**

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