Tumor-suppressive signaling in the uterus

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Endometrial cancer is the most common gynecologic cancer, with 63,230 new cases and 11,350 deaths in the United States in 2018 (1). The resulting fertility loss and the life-threatening potential of uterine cancer are devastating. Unfortunately, the etiology of endometrial cancer remains poorly defined, hampering the design of effective diagnostic and therapeutic options for this disease. In PNAS, two exciting studies from the Matzuk laboratory (2, 3) elegantly demonstrate that transforming growth factor β (TGF-β) signaling maintains endometrial homeostasis by suppressing cell overgrowth and oncogenesis. This action, the researchers show, is achieved through the TGF-β type I receptor (TGFBR1, or ALK5) and SMAD2/3. The findings significantly advance our understanding of the development of uterine cancer.

TGF-β signaling regulates fundamental cellular functions. The canonical pathway involves ligands, receptors (types I and II), and SMAD transducers (4). SMAD2/3 and SMAD1/5/8 commonly mediate cell signaling from TGF-βs/activins and bone morphogenetic proteins (BMPs), respectively (4). Because various signaling components are mutated or otherwise altered in human disease (5), this pathway is an attractive therapeutic target. The TGF-β signaling pathway is critical for cancer development (6). Although mutation of the pathway components accounts for only a very small percentage of endometrial cancer, alteration in the signaling activity may be a common contributing factor (7–12). To determine whether TGF-β signaling is involved in uterine physiology and cancer development, the Matzuk team took advantage of the Cre-loxP approach by generating mice with conditional knockout (cKO) of *Alk5* [Monsivais et al. (2)] or *Smad2/3* [Kriseman et al. (3)] in the uterus (*Alk5* cKO or *Smad2/3* cKO mice, respectively). The researchers used progesterone receptor (*Pgr*) Cre recombinase, which deletes genes in uterine epithelial, stromal, and myometrial cells (2, 3). A major strength of these studies lies in the use of a genetic approach that conditionally disrupts two functionally related signaling molecules in the TGF-β pathway. The involvement of ALK5 and SMAD2/3 in reproductive development and ovarian tumorigenesis has been demonstrated using loss-of-function (13, 14) and/or gain-of-function mouse models (15, 16). However, the role of TGF-β signaling in endometrial cancer development remains elusive. The reports by Monsivais et al. (2) and Kriseman et al. (3) in PNAS represent a major step toward finding the missing piece of the puzzle.

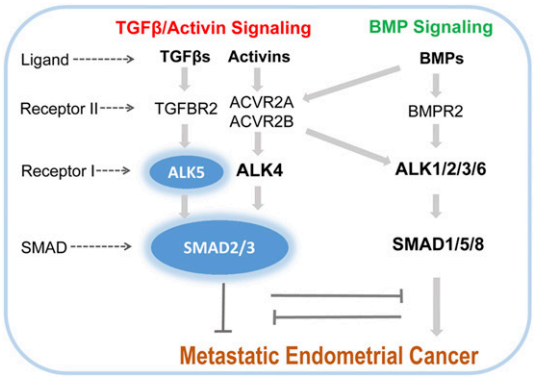

Fig. 1. Proposed model of cell signaling associated with the ALK5-SMAD2/3 tumor-suppressive arm identified by Monsivais et al. (2) and Kriseman et al. (3) in the uterus. TGF-βs and activins signal through TGFBR2/ALK5 and ACVR2A/ACVR2B/ALK4 receptor complexes, respectively. In contrast, BMPs signal through BMPR2/ACVR2A/ACVR2B and ALK1/2/3/6 receptors, which impinge on SMAD1/5/8. Because cell signaling initiated both by activins and TGF-βs converges on SMAD2/3, ALK4 and ALK5 may play redundant roles in endometrial cancer development. The existence of antagonisms between tumor-suppressive TGF-β and oncogenic BMP signaling is supported by increased expression of BMP target genes in *Smad2/3* cKO uteri (3). It should be pointed out that the ligand-induced cell signaling, receptor utilization, and pathway interactions shown in this model require further experimental testing.

The loss of growth inhibitory function of TGF-β signaling in endometrial cancer is well documented (11). However, genetic evidence that directly links TGF-β signaling inactivation to endometrial carcinogenesis is lacking. If TGF-β signaling inhibits tumorigenesis in

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the uterus, does ablation of the type I receptor ALK5 lead to malignant transformation? Monsivais et al. (2) report that *Alk5* cKO mice develop metastatic endometrial tumors. It should be emphasized that loss of ALK5 promotes endometrial oncogenesis only in female mice being continuously mated with fertile males, consistent with our previous finding that nulliparous female *Alk5* cKO mice are cancer-free (17). Notably, the main metastatic site of endometrial cancer in *Alk5* cKO mice is the lung, which contains a recombined *Alk5* allele (2). We previously disrupted *Alk5* in phosphatase and tensin homolog (PTEN)-depleted endometrium (17). Loss of ALK5 exacerbates progression of endometrial cancer induced by PTEN inactivation and promotes pulmonary metastasis (17). A more recent study using mice with uterine epithelial cell-specific deletion of *Pten* also suggests a tumor-suppressive function of uterine TGF-β signaling (18). Monsivais et al. (2) extend the previous findings (17) by showing that ablation of ALK5 per se can lead to the development of metastatic endometrial cancer, contingent on a second event of pregnancy/parturition-induced endometrial remodeling. This exciting observation inspired further experimentation using a unilateral oviduct ablation model, in which only uterine horns with an intact oviduct develop cancer (2). This discovery indicates that ALK5-mediated signaling helps maintain the structural and functional integrity of the endometrium after pregnancy/parturition-induced cellular remodeling.

Because SMAD2/3 mediates TGF-β and activin signaling, does loss of SMAD2/3 lead to endometrial cancer formation? Kriseman et al. (3) generated and characterized the *Smad2/3* cKO mouse model and provide additional evidence supporting the role of canonical TGF-β signaling in uterine cancer development. Endometrial hyperplasia is an early phenotype of these mice (3). Supporting the precancerous feature of this condition, *Smad2/3* cKO mice ultimately develop endometrial cancer (3). Therefore, the *Smad2/3* cKO mouse model may help identify mechanisms underpinning malignant transformation of endometrial hyperplasia.

The major conceptual advance reported in the two papers in PNAS (2, 3) is the discovery of an ALK5-SMAD2/3 tumor-suppressive arm of TGF-β signaling in the uterus (Fig. 1). Monsivais et al. (2) additionally uncover a previously unrecognized, pregnancy-dependent role of ALK5 in uterine cancer development. Using the *Smad2/3* cKO model, Kriseman et al. (3) have garnered a wealth of information on transcriptomic alterations in endometrial cancer. Mechanistically, cancer development in *Smad2/3* cKO mice appears to involve the interplay between TGF-β and BMP signaling, with loss of SMAD2/3 promoting the expression of BMP target genes associated with cell growth and angiogenesis (3) (Fig. 1). Using RNA-sequencing, Kriseman et al. (3) also identify altered genes involved in steroid biosynthesis, cell motility, and the tumor microenvironment. Despite these important findings, it is unclear how loss of SMAD2/3 affects BMP signaling. Perhaps SMAD2/3 directly suppresses the BMP pathway, or perhaps BMP signaling is up-regulated to compensate for the loss of SMAD2/3. Ablation of SMAD2/3 may also affect the BMP pathway, because both activins and BMPs share ACVR2A/ACVR2B (19) (Fig. 1).

What are the implications of the findings from the Matzuk team (2, 3)? Conceptually, these findings may open a new avenue for developing treatment modalities for endometrial cancer. The transcriptomic data from Kriseman et al. (3) could be exploited to identify novel diagnostic and therapeutic targets that control cell proliferation, the tumor microenvironment, metastasis, and steroid biosynthesis. Steroid hormones are systemic modulators of reproductive function and play key roles in gynecologic cancers. The diminished expression of PGR in the cancerous epithelium of *Alk5* cKO and *Smad2/3* cKO uteri (2, 3) indicates dysfunctional hormone signaling. Indeed, removal of the ovaries promotes the regression or prevents the formation of endometrial cancer in these models, although the timing of ovariectomy appears to be critical (2, 3). Because of the impaired uterine PGR expression, the *Alk5* cKO and *Smad2/3* cKO mouse models may be useful in exploring therapeutic strategies for PGR-negative endometrial cancer. Theoretically, effective therapy for metastatic endometrial cancer could be achieved through the following: (*i*) strengthening the ALK5-SMAD2/3 tumor-suppressive arm in cancers with compromised TGF-β signaling activity; (*ii*) counteracting tumor-promoting mechanisms (e.g., enhanced BMP signaling) resulting from dysregulated ALK5-SMAD2/3 signaling; and (*iii*) restoring progesterone responsiveness in endometrial cancer cells with impaired progesterone signaling or a hormonal imbalance. However, caution should be exercised in extrapolating these findings to human endometrial cancer, as the mouse uterus and human uterus differ regarding uterine anatomy, endocrinology of the estrous cycle, and uterine responses to estrogen (20).

The reports by Monsivais et al. and Kriseman et al. unambiguously reveal ALK5-SMAD2/3 as a potent tumor-suppressive arm of TGF-β signaling in the mammalian uterus.

Besides potential therapeutic value, the discoveries by Monsivais et al. (2) and Kriseman et al. (3) raise interesting questions that deserve further investigation. Although both *Alk5* cKO and *Smad2/3* cKO mice develop metastatic endometrial cancer, differences exist in the onset of endometrial lesion and dependence on pregnancy/parturition-associated events (2, 3). These differences suggest that there are distinct signaling circuits intertwined with ALK5 or SMAD2/3. Further research is needed to unveil the oncogenic cascades triggered by ablation of ALK5. Comparative analysis of genes regulated by ALK5 and SMAD2/3 signaling may provide new insights into this question. Because ALK5 and SMAD2/3 are deleted in both epithelial and stromal compartments of the uterus, it would be interesting to determine whether epithelial–stromal interactions are involved in uterine cancer development. And because SMAD2/3 can mediate both activin and TGF-β signals transmitted through the respective ALK4 and ALK5 (Fig. 1), it would be important to test the potential redundancy between these receptors and determine whether mice with targeted deletion of both receptors phenocopy *Smad2/3* cKO mice. In addition, what are the roles of activins or other ligands that are functionally associated with SMAD2/3 in the *Smad2/3* cKO model? How does loss of ALK5-SMAD2/3 signaling promote metastasis of cancer cells? Further elucidating the TGF-β family signaling landscape will advance our understanding of how ALK5 and SMAD2/3 interact with other pathways to control endometrial homeostasis and function, and how their dysregulations render endometrial cells susceptible to oncogenic insult.

In summary, the reports by Monsivais et al. (2) and Kriseman et al. (3) unambiguously reveal ALK5-SMAD2/3 as a potent tumor-suppressive arm of TGF-β signaling in the mammalian uterus. We are now one step closer to understanding the role of the master growth factor signaling pathway of TGF-β in the

pathogenesis of endometrial cancer. The mouse models in refs. 2 and 3 may be harnessed to improve the treatment of endometrial cancer. The findings also raise important questions about the mechanisms of TGF-β signaling in uterine cancer development that merit future investigation. The knowledge stemming from such investigation may have broad relevance in cancer management.

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1 Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. *CA Cancer J Clin* 68:7–30.
2 Monsivais D, Peng J, Kang Y, Matzuk MM (2019) Activin-like kinase 5 (ALK5) inactivation in the mouse uterus results in metastatic endometrial carcinoma. *Proc Natl Acad Sci USA* 116:3883–3892.
3 Kriseman M, et al. (2019) Uterine double-conditional inactivation of *Smad2* and *Smad3* in mice causes endometrial dysregulation, infertility, and uterine cancer. *Proc Natl Acad Sci USA* 116:3873–3882.
4 Li Q (2014) Transforming growth factor β signaling in uterine development and function. *J Anim Sci Biotechnol* 5:52.
5 Gordon KJ, Blobe GC (2008) Role of transforming growth factor-β superfamily signaling pathways in human disease. *Biochim Biophys Acta* 1782:197–228.
6 Massagué J, Blain SW, Lo RS (2000) TGFbeta signaling in growth control, cancer, and heritable disorders. *Cell* 103:295–309.
7 Sakaguchi J, et al. (2005) Aberrant expression and mutations of TGF-beta receptor type II gene in endometrial cancer. *Gynecol Oncol* 98:427–433.
8 Mhawech-Fauceglia P, et al. (2011) Expression and clinical significance of the transforming growth factor-β signalling pathway in endometrial cancer. *Histopathology* 59:63–72.
9 Eritja N, et al. (2017) Endometrial carcinoma: Specific targeted pathways. *Molecular Genetics of Endometrial Carcinoma*, ed Ellenson LH (Springer International Publishing, Cham, Switzerland), pp 149–207.
10 Piestrzeniewicz-Ulanska D, McGuinness DH, Yeaman GR (2008) TGF-β signaling in endometrial cancer. *Transforming Growth Factor-β in Cancer Therapy, Volume II: Cancer Treatment and Therapy*, ed Jakowlew SB (Humana Press, Totowa, NJ), pp 63–78.
11 Parekh TV, et al. (2002) Transforming growth factor beta signaling is disabled early in human endometrial carcinogenesis concomitant with loss of growth inhibition. *Cancer Res* 62:2778–2790.
12 Kandoth C, et al.; Cancer Genome Atlas Research Network (2013) Integrated genomic characterization of endometrial carcinoma. *Nature* 497:67–73.
13 Li Q, et al. (2008) Redundant roles of SMAD2 and SMAD3 in ovarian granulosa cells in vivo. *Mol Cell Biol* 28:7001–7011.
14 Li Q, et al. (2011) Transforming growth factor β receptor type 1 is essential for female reproductive tract integrity and function. *PLoS Genet* 7:e1002320.
15 Gao Y, et al. (2016) Constitutively active transforming growth factor β receptor 1 in the mouse ovary promotes tumorigenesis. *Oncotarget* 7:40904–40918.
16 Gao Y, et al. (2015) Constitutive activation of transforming growth factor Beta receptor 1 in the mouse uterus impairs uterine morphology and function. *Biol Reprod* 92:34.
17 Gao Y, Lin P, Lydon JP, Li Q (2017) Conditional abrogation of transforming growth factor-β receptor 1 in PTEN-inactivated endometrium promotes endometrial cancer progression in mice. *J Pathol* 243:89–99.
18 Liang X, et al. (2018) The uterine epithelial loss of Pten is inefficient to induce endometrial cancer with intact stromal Pten. *PLoS Genet* 14:e1007630.
19 Pardali E, Goumans MJ, ten Dijke P (2010) Signaling by members of the TGF-beta family in vascular morphogenesis and disease. *Trends Cell Biol* 20:556–567.
20 Groothuis PG, Dassen HH, Romano A, Punyadeera C (2007) Estrogen and the endometrium: Lessons learned from gene expression profiling in rodents and human. *Hum Reprod Update* 13:405–417.