Clinical Cancer Research

Targeting TGFβ Pathway in Adult Granulosa Cell Tumor: Opening Pandora's Box?

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Adult granulosa cell tumor (AGCT) is a rare malignancy characterized by *FOXL2* (*C134W*) mutation, an inherent component of the TGFβ pathway. Activin A, a TGFβ superfamily member, is a driver of this activity and is a potential target in AGCT.

See related article by Tao et al., p. 5458

In this issue of *Clinical Cancer Research*, Tao and colleagues (1) report the soluble receptor ligand trap targeting activin A, STM 434, achieves dose-related metabolic effects without evidence of antitumor efficacy, including in 13 patients with AGCT. The tumor-suppressive function of TGFβ can be lost due to accumulation of genetic alterations in critical pathway members (TGFBR2, TGFBR1, SMAD2, SMAD4/DPC4) and pathway deactivation leads to tumor promotion. Simultaneously, microenvironment components express TGFβ receptors responsible for modulating angiogenesis, immune cell tumor infiltration and fibrosis, which support tumor development and metastasis. Crosstalk between the microenvironment and tumor cells stimulates secretion of growth factors EGF, IGF-1, PDGF, FGF, MMP, and type I collagen, which results in facilitating tumor growth, invasion, and metastasis (Fig. 1; refs. 2, 3). The main effect of TGFβ targeting may be mediated by microenvironmental effects (4) and combination trials with chemotherapy, immunotherapy, and antiangiogenic agents have been proposed. There is a quest to develop predictive biomarkers and to targeting increasingly specific mediators of TGFβ activity.

Activin is a hydrophilic nonsteroid protein mainly synthesized by pituitary and ovarian granulosa cells. Its function is activated by binding to activin-type 1 and 2 receptors, which together as a complex activate SMAD2/3 through phosphorylation, leading to an increase in the granulosa cell population during folliculogenesis (5). This is exemplified by the current trial, targeting activin A, a known member of the TGFβ superfamily. This effect can be inhibited by follistatin, an activin-binding protein that induces atresia of large antral follicles in mammals. Coordination of protein function is regulated by *FOXL2;* a transcription factor that is fundamental in sex determination (4). *FOXL2* is responsible for folliculogenesis, germ cell proliferation, and overall maintenance of the ovarian phenotype through life (6).

Shah and colleagues (2009) reported a single, recurrent somatic mutation *402C>G* in *FOXL2*, *FOXL2 (C134W)*—present in almost all morphologically identified in AGCT, but not in juvenile GCT—describing this mutation as a key pathogenic driver (7). Although it is evident that *FOXL2* (*C134W*) is directly involved in the pathogenesis of AGCT, this mutation has dual characteristics of oncogene and tumor suppressor gene, depending on GCT subtype genetic context. As *FOXL2 (C134W)* mutation conserves some of the wild-type gene transcriptional functions, there are three main routes that promote tumorigenesis in granulosa cells including: (i) deregulation of TGFβ antiproliferative pathway; (ii) reduction in apoptosis with decreased expression of cell death mediators TNF-R1 and FAS; and (iii) reduction in GnRH-induced apoptosis. *FOXL2* (*C134W*) mutation also causes abnormal steroidogenesis by disrupting the FOXL2-SF-1 complex function, which is responsible for mediating CYP19 transcription (P450 aromatase) required to regulate the conversion of androgen to estrogen (8).

AGCTs represent approximately 2%–5% of ovarian malignancies and are the most frequent ovarian sex cord/stromal tumor (9), associated with a long-term natural history and slow disease growth. Surgery remains the most successful treatment approach; yet, approximately 30% of patients with early-stage disease will relapse after primary debulking surgery (10) and eventually die from complications of disease progression. Effective therapeutic options are limited and have to balance disease control with an acceptable toxicity profile. With this in mind, targeting activin A is logical, as a driver of AGCT proliferation and a consequence of the TGFβ pathway disruption caused by *FOXL2* (*C134W*) mutation.

In the current publication, a first in human phase I study that assessed the activin A inhibitor, STM 434, to define the MTD, as well as to determine safety and antitumoral activity in 32 patients receiving different doses. Thirteen AGCT patients were enrolled onto the study, considered to be refractory, intolerant of standard treatment, or not to have standard treatment available. Although no median number of treatment lines or type of treatment used previous to STM 434 exposure were described, in total, 1–11 treatment lines were given before exposure to STM 434. In this cohort, 10 of 12 patients with AGCT achieved stable disease as best response with very short median duration of therapy of 7.1 weeks. Although the safety profile showed mainly grade 1 and 2 toxicities, a significant proportion of patients (53%) experienced bleeding episodes. Grade 3 toxicities included epistaxis, abdominal pain and anemia in 3%, 6% and 6%, respectively. Five DLTs were documented in 3 patients. These results show limited AGCT antitumoral activity, and without convincing disease stabilization (median duration 7.1 weeks), which did not appear to correlate with dose level exposure. Given the usual slow course of

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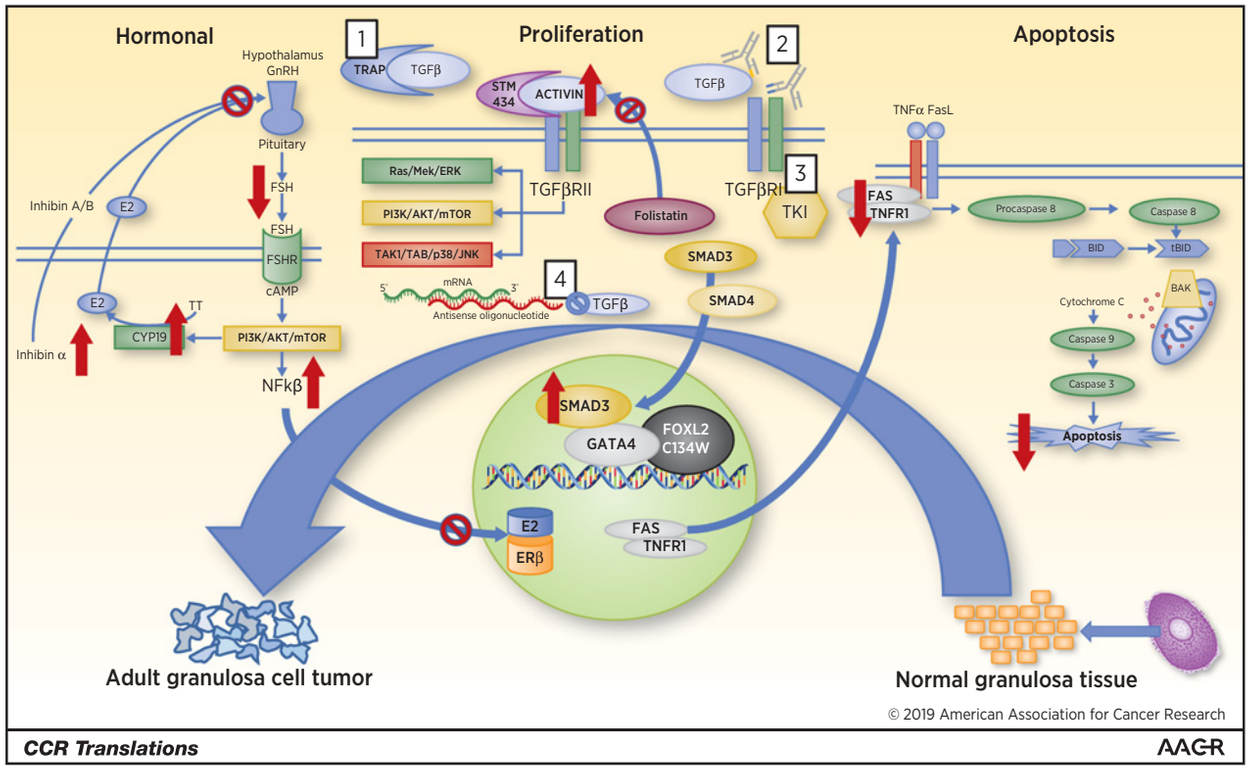

Figure 1.

Adult granulosa cell tumor (AGCT) pathogenesis and TGFβ-targeting molecules. *FOXL2 (C134W)* mutation represents the unique and highly frequent genetic alteration in AGCT, leading AGCT pathogenesis, trough alteration in cell proliferation, decrease in apoptosis, and altered steroidogenesis. *FOXL2 (C134W)* pathologic cell proliferation is led by TGFβ pathway. Activin A, a TGFβ superfamily member, is a driver of this activity and a potential target in AGCT. STM 434, a soluble receptor ligand trap, targets activin A. Other molecules targeting TGFβ pathway include: 1, Ligand-traps, TGFβ-neutralizing soluble receptors like M7824. 2, Monoclonal antibodies, targeting TGFβ ligands and TGFβ receptors, such as GC1008, a pan-TGFβ antibody, 1D11, 2G7 and LY3022859, a mAb against TGFβRII. 3, Small-molecule inhibitors, SB431542, KI26894, SD208, IN-1130, LY210976, and LY2157299 inhibitor of TGFβRI. 4, Antisense oligonucleotides, that decrease TGFβ ligand mRNA synthesis, AP12009, targeting TGFβ2, AP11004, and AP15012, a TGFβ2 antisense gene–modified allogeneic cancer cell vaccine.

AGCT progression, it is perhaps optimistic to attribute disease stabilization to this population to STM 434 exposure in the absence of a control; however, this trial represents an important attempt at targeting activin A, an essential factor in the pathogenesis of AGCT. Study findings here create the opportunity to examine how to successfully target TFGβ pathway disruption in patients with AGCT. *FOXL2 (C134W)* mutation has a duality of function whereby it may operate as a tumor suppressor gene or an oncogene depending the genetic context (11, 12). Although the mutation is present, most functions remain intact and localized within the nucleus as no cytoplasmatic translocation is evident (13); however, this may result in an intermittent rather than permanent disruption in the TGFβ pathway. *TGFβ* by itself carries bivalent characteristics of tumor suppressor or promotor depending on the stage of tumor evolution, in addition to the complexity and variability of crosstalk between TGFβ canonical—modulating gene expression to produce the physiological and pathological activities of TGFβ—and non-canonical pathway, generating signaling through TNF receptor-associated factor 4 (TRAF4), TRAF6, TGFβ-activated kinase 1 (TAK1; known as MAP3K7), p38 MAPK, RHO, PI3K, AKT, ERK, JUN N-terminal kinase (JNK), or nuclear factor-κB (NF-κB), ultimately define TGFβ activity consequences. AGCT pathogenesis is complex, probably due to pleiotropic functions of TGFβ in tumorigenesis.

Hormone treatment as the systemic standard therapy of choice is also a targeted therapy (14), and in the setting of AGCT, its activity is probably related to abnormal steroidogenesis caused by *FOXL2* (*C134W*) mutation. Despite efficacy of hormonal therapy in AGCT, patients with recurrent disease eventually progress and become resistant to these treatments. Given usual prolonged and slow AGCT disease course, it is necessary to find alternative ways to treat disease with novel agents that are effective, well tolerated and perhaps suitable for chronic administration. Our search toward identifying and exploiting therapeutic vulnerabilities in AGCT need to continue, and build in tissue based pharmacodynamic assessment and Tao and colleagues are to be congratulated for bringing biologically based evaluation to this rare disease.

Disclosure of Potential Conflicts of Interest

A. M. Oza is a consultant/advisory board member for Immunogen, AstraZeneca, Tesaro, and Clovis. No potential conflicts of interest were disclosed by the other author.

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