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Review Article

# Transgenic mice expressing inhibin α-subunit promoter (inhα)/Simian Virus 40 T-antigen (Tag) transgene as a model for the therapy of granulosa cell-derived ovarian cancer

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ABSTRACT

Granulosa cell tumors are rare, 3–7.6% of primary ovarian tumors, although with poor prognosis as the tumor-related mortality rate is 37.3%, with 80% of deaths occurring on recurrence. We have created a transgenic (TG) murine model for gonadal somatic cell tumors by expressing the powerful viral oncogene, Simian Virus 40 T-antigen (Tag), under the regulation of murine inhibin α-subunit 6 kb promoter (inhα/Tag). Gonadotropin dependent ovarian granulosa cell tumors were formed in females by the age of 5–6 months, with a 100% penetrance. We have successfully used the inhα/Tag model to test different treatment strategies for ovarian tumors. With a gene therapy trial in inhα/Tag mice crossbred with inhα/HSV-TK (herpes simplex virus thymidine kinase) mice (double TG), we proved the principle that targeted expression of HSV-TK gene in gonadal somatic cell tumors enabled tumor ablation by anti-herpes treatment. When we aimed at targeted destruction of luteinizing hormone/chorionic gonadotropin receptor (LHCGR) expressing inhα/Tag tumor cells *in vivo* by a lytic peptide Hecate-CGβ conjugate, we could successfully kill the tumor cells, sparing the normal cells. We recently found high zona pellucida glycoprotein 3 (ZP3) expression in inhα/Tag granulosa cell tumors, as well as in human granulosa cell tumors. We tested the concept of treating the ovarian tumors of inhα/Tag mice by vaccination against the ectopically expressed ZP3. Immunotherapy with recombinant human (rh) ZP3 was highly successful with no objective side effects in

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inhα/Tag females, suggesting rhZP3 immunization as a novel strategy for the immunotherapy of ovarian granulosa cell tumors.



## 1. Introduction

Ovarian cancer is the third most common type of gynecological malignancy (after cervical and endometrial cancer), although with the highest mortality rate in the female reproductive system [1]. Ovarian tumors originate from three cell types. Germ cell tumors of the ovary are uncommon (~3%), but aggressive tumors are mostly seen among young women or adolescent girls, frequently unilateral, and are generally curable if diagnosed and treated early [2]. Ovarian sex cord-stromal tumors are a heterogeneous group of benign or malignant tumors derived from stromal components that comprise of the granulosa cells, theca cells and fibrocytes [3]. This group accounts for ~7% of ovarian cancers, which occur in women of all ages, has a prognosis and tumor-related mortality rate of 37.3%, where ~80% of deaths occur on recurrence [4]. Surface epithelium-derived tumors are the largest group (~90%) of ovarian tumors, with 50% of all cases occurring in women older than 65 years [5]. For surface epithelial and stromal tumors, the most common sites of metastasis are the pleural cavity (33%), liver (26%), and lungs (3%) [6–8].

## 2. Granulosa cell tumor

Based on clinical behavior and histopathological findings granulosa cell tumors (GCT) can be divided into two subtypes: adult (AGCT; 95%) and juvenile (JGCT; 5%) [9]. Primary treatment for patients with stage I of GCTs is surgery. Postoperative treatment options such as chemotherapy, radiotherapy or hormonal treatment are often considered to be reasonable choice for patients with advanced disease by surgical staging, and for patients with recurrent tumor [9–11].

Many genetic and hormonal risk factors that contribute to the development of GCT have been identified. Major genetic risk factors are associated with cytogenetic abnormalities (chromosomal aberrations with trisomy 12, trisomy 14, and monosomy 22) [12,13], and genetic syndromes such as Peutz-Jeghers syndrome, Ollier disease and Maffucci syndrome [14,15]. Among hormonal risk factors leading to GCT, hyper stimulation with ovulation-inducing drugs such as the selective estrogen receptor modulator (SERM), clomiphene citrate, or exposure to high concentrations of gonadotropins in the context of infertility treatments [16–18] was shown. A recent study has linked a missense point mutation (C402C-G) in *FOXL2* gene (a member of the forkhead–winged helix family of transcription factors) to GCT [19]. Independent sequencing analysis of GCT samples revealed that this mutation was highly specific for AGCT (272/289, 94%) and very rare in JGCT (2/31, 6%) [10]. Under physiological conditions, FOXL2 is required for the normal granulosa cells differentiation and ovary maintenance [20]. Given that estradiol, inhibin B and anti-Müllerian hormone (AMH) are not fully reliable tumor markers (reviewed in [10]), screening for *FOXL2* mutation could become an important diagnostic tool to identify and differentiate between adult and juvenile GCTs.

GCT research could highly benefit from animal/murine models, especially the research on their molecular pathogenesis and to test *in vivo* novel treatment strategies. To date there are very few murine models available for GCT research. Female mice of SWR related strains develop spontaneous juvenile type ovarian GCT around 4–6 weeks of age with rather low 2% (SWR), 15% (SWXJ-9 recombinant inbred) and 27% (SWRxSWXJ-9 F1 hybrids) incidence rates [21,22]. The number of transgenic (TG) mouse models for GCT is also limited and the existing models are only partially relevant to humans. Concerning genetically modified mouse models, disruption of activin and bone morphogenic protein receptors (BMPRs) [23], *inhibin α* [24], SMADs [25], and nuclear hormone receptors such as *Esr2* [26] or *Wnt/β-catenin/Ctnnb1* and *Pten*, have been found to lead to GCT [27–29].

## 3. Inhibin α-SV40 Tag transgenic mice

We have created transgenic mice carrying the SV40 large T-antigen under the inhibin α promoter (Inhα/Tag) [30,31] for tissue-selective gonadal somatic cell tumors (GCT in females and Leydig cell tumors in males) (Fig. 1). These mice develop gonadal tumors, with 100% penetrance by the age of 5–7 months (Fig. 1) [30,31]. Another important feature of the Inhα/Tag mice is that when prepubertally gonadectomized they develop adrenocortical tumors with 100% incidence [32,33] (Fig. 1). Leydig and adrenocortical tumors in Inhα/Tag will not be addressed in this brief review article.

Inhα/Tag females present decreased circulating levels of gonadotropins (LH and FSH) while serum free inhibin α subunit, inhibin B and progesterone levels increase along with tumor growth [30,31,34,35]. Gonadal tumorigenesis in Inhα/Tag is gonadotropin dependent and tumor cells express LHCGR abundantly. Gonadal tumorigenesis was blocked while the Inhα/Tag mice were treated with either GnRH antagonist or crossbreed with the gonadotropin-deficient hypogonadal mutant (*hpg*) genetic background mice [36]. The Inhα/Tag GCTs metastasize to liver and lungs, thus resembling human ovarian GCTs [34]. Cell lines derived from gonadal tumors of these mice (KK-1 and NT-1 from females and BLT-1 and BLTK-1 cell lines from males) retain their steroidogenic activity and gonadotropin receptors and gonadotropin responsiveness [30]. Young Inhα/Tag females up to 12 weeks (before tumor development) shows normal estrous cycles and produce