

Gonadal Tumors Developed from Consecutively Transplanted Spleens Bearing Gonad Implants

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This study was conducted by consecutively transplanting spleens, which had gonads implanted previously. A total of 84 cases for infantile testicles and 106 cases for ovarian follicles were performed. In the case of ovarian implants, the results were determined by the total number of follicle implants. A modified spleen transplantation technique called double implantation of ovarian follicles was applied to increase the amount of the implants. In this technique, an extra spleen is implanted into the potential donor so that the ovarian follicles can be implanted to two different spleens, doubling the amount of implants.

Through consecutive spleen transplantation, we observed the results beyond a typical rat's life span. In many of these cases, we found more aggressive forms of malignant tumor, seminoma and dysgerminoma. We present the results and discuss possible pathogenic mechanisms of tumor formation.

Key Words: Consecutive spleen transplantation, testicle and ovary implant, seminoma, dysgerminoma

INTRODUCTION

The results of consecutive spleen transplantation bearing gonads in rats were presented previously in two articles by this laboratory.^{1,2} Several kinds of tumors developed from infantile testicle implants in young castrates, especially

malignant tumors such as seminoma-like transformation, could be seen after increasing total duration of implantation into spleen for over 26-27 months.¹ We could observe similar results in female rats by inducing tumor growth in the spleens from the mature ovarian follicle implants and consecutively transplanting them into the next young castrates.² Similar to Biskind's observations,^{3,4} many of the spleen tumors showed benign sex-cord stromal tumors, such as granulosa or granulosa-theca cell tumor and Sertoli or Sertoli-Leydig cell tumor even though the implantation period was extended to 25 months.

Since 1998 we have studied the result of the consecutive spleen transplantation after implanting the infantile testicle and one ovarian follicle. From 2000 on, the outcomes of ovarian tumor were studied with different modes of implantation. The total number of ovarian follicles implanted was increased to five and then to ten, using the double implantation of ovarian follicles (DIO) technique.² Many of the tumors developed into dysgerminoma in these rats at 11.5-14 months after DIO, which was in relatively shorter period than other total implant duration.

The increased time of exposure and amount of gonads implanted are possible etiologic factors for malignancy development in these experimental models. We present the results of the variable gonad tumors developed from their implants in this article.

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MATERIALS AND METHODS

For testicular implant study, infantile inbred Lewis rats that were less than 10 days old were used as donors, and 3 month-old male inbred Lewis rats weighing 150-200 grams were used as recipients for implant or spleen transplantation.

We implanted one entire immature Lewis testicles, including the epididymal elements, under the capsule of the spleen of 3 month-old castrate through two small nicks as described in previous papers to induce tumor growth.^{1,5} After 9-15 months the spleens bearing various testicular tumors were transplanted to the next young castrated generations (1SPT), as shown in Fig. 1A. The procedure was repeated consecutively to the next generations, creating 2SPT and 3SPT groups (Table 1). With these methods we extended the total duration of testicular implant to maximum of 43 months.

For ovarian implant study, 2.5-3 month old female inbred Lewis rats weighing 150-170 grams

were used as both donors for the ovarian or spleen, and recipients for implant or spleen transplantation. One or five mature follicles from the one side of the ovaries were inserted into the pouch formed in the spleen, and the opening was closed with suture, as described in previous papers, to induce tumor growth.^{2,5} Total of ten follicles were also implanted using the DIO technique. The DIO technique is first performed by procuring the spleen from a donor female rat and transplanting it into a recipient. Then five mature ovarian follicles are implanted into each of the spleens. After 9-15 months, the spleens harboring ovarian tumor were examined, and transplanted to the next young female castrates (1SPO). This procedure was repeated consecutively to create 2nd and 3rd generations (2SPO and 3SPO, respectively) as previously reported.² For 5 years, we implanted a total of 106 cases of one or five ovarian follicles and total of ten follicles (Table 1). 11 cases of the modified DIO group had five more mature ovarian follicles implanted into the native or transplanted spleen with already-grown ovarian tumors, as shown in Fig. 1B.

For splenic transplantation, splenic artery-celiac-aortic segment and splenic vein-portal venous segment from the donors were isolated in en-bloc style, and end-to-side anastomosed with the aorta and portal vein of the recipients, respectively, using microvascular surgical technique.⁶ All recipient rats were castrated. All surgical procedures were conducted in a humane manner under ether anesthesia using clean technique, according to the National Research Council's Guidelines for the use of laboratory animals.

We examined the pathologic specimens from both testicular and ovarian follicle implant cases according to our laboratory protocol. The total duration of the implantations were variable for each implants; testicles from 9 to 43 months and ovaries from 9 to 34 months.

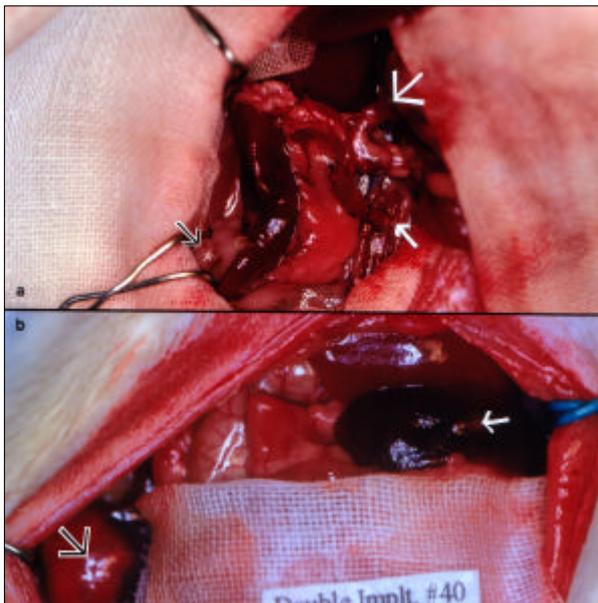


Fig. 1. a. Transplantation of spleen bearing tumor (1SPT). Testicular tumor in the lower portion of spleen 14 months after implantation (black arrow). Arterial (white small arrow) and portal venous (white big arrow) anastomosis between graft and host. b. Double implantation of ovary (DIO) + 1SPO + Add. Imp. Ovarian tumor grown in the previous implanted (native) spleen through DIO after 12 months, and then consecutively transplanted (big black arrow). Newly implanted five ovarian follicles into the native spleen (small white arrow).

RESULTS

A total of 38 cases of spleen transplantations were successfully done in the first generation after the testicular tumor development in the spleen (1SPT). The spleens had variously sized and

Table 1. Results of the Gonad Implant into Spleen and Consecutive Spleen Transplantation

Gonad Implant	Mode of Implantation	Case	Range* of Implant Duration (Ovary Age)	Pathology [†]	
				Gonadal Stromal Tumor (G)	Dysgerminoma (D) Seminoma (S)
Testicle	Implantation of 1 Infantile testicle into Spleen	84	9 - 16		
	1 SPT	38	16.5 - 26.5	10/11: G	
	2 SPT	9	25 - 30.5	4/5: S	
	3 SPT	1	43	1/1: S	
Ovary	Implantation of 1 - 10 follicles into Spleen	106	9 - 15 (11.5 - 18)		
	1 SPO				
	-1 follicle		18.5 - 25	5/7: G	
	-5 follicles	33	(21 - 27.5)	5/6: D	
	2 SPO				
	-1 follicle		25 - 27.5	2/2: G	
	-5 follicles	5	(28 - 30)	3/3: D	
	3 SPO		21, 26		
	-5 follicles	2	(25, 32)	2/2: D	
	DIO + 1 SPO [‡]	6	16.5 - 28.5 (20 - 31)	5/6: D	
DIO + 1 SPO + Add. Imp [§]	8	15 - 26.5 (19 - 30)	3/3: D		
DIO + 2 SPO + Add. Imp [§]	3	25, 26, 34 (31, 32, 41)	2/3: D		

*Expressed as months, each gonad age in the case of testicular implantation is the same as each implant duration. Tx, Transplantation. Each testicular age is the same as the total implant duration, while ovarian age is usually 2.5 - 7 months different from the total implant duration.

[†]Pathology examined in limited numbers: each denominator represents total cases of biopsy.

1 SPT: Transplantation of spleen bearing testicular tumor in the 1st generation.

2(3) SPT: Consecutive transplantation of spleen from 1(2) SPT to the 2nd (3rd) generation.

1 SPO: Transplantation of spleen bearing ovarian tumor in the 1st generation.

1 SPO-1 (-5) follicles: 1 SPO performed with the tumor that arose from the spleen implanted with one follicle (or five follicles) that was (those were) previously implanted into spleen.

2(3) SPO: Consecutive transplantation of spleen from 1(2) SPO to the 2nd (3rd) generation.

[‡]DIO: Double implantation of each five ovarian follicles, therefore ten ovarian follicles in total.

[§]Add. Imp: After transplantation of spleen bearing ovarian tumor, five ovarian follicles were added more into native or grafted spleen. (As of Dec/31/2003)

shaped tumors, ranging from 1.5cm to 2.5cm in diameter. Among the 1SPT group, 9 were consecutively transplanted into the next generation (2SPT). This group enabled us to observe the tumor for maximum 30.5 months as of December 2003. We examined 17 pathologic specimens. Most frequently, 11 specimens of the 1SPT group were

benign sex-cord stromal tumors. However, in the consecutively transplanted 2SPT group, many were malignant type of tumor - seminoma. One case of the oldest showed the same result (Table 1).

A total of 33 cases of successful spleen transplantation were done in the first generation after the ovarian tumor development in the spleen

(1SPO). The spleens had various tumors as a result of 1 or 5 ovarian follicles implantation. 5 spleens from 33 transplanted spleens were consecutively transplanted into the next generation (2SPO), allowing us to observe the tumor for maximum 27.5 months as of December 2003. 32 cases of the successfully transplanted spleens were examined pathologically. Of the 7 cases of ovarian tumor from one-follicle implanted spleens those were transplanted in the first generation (1SPO-1 follicle), 5 were gonadal stromal tumor. However, 5 of the 6 cases of tumor from five-follicle implanted spleens those were transplanted (1SPO-5 follicles) in the same generation as one-follicle implanted spleens were dysgerminoma (Table 1).

3 cases of ovarian tumor arose from 5 follicles implanted and consecutively transplanted spleens to the second (2SPO-5 follicles), or in 2 cases, to the third generations (3SPO-5 follicles), with the implant duration extending to 21 - 27.5 months, they also showed the same malignant result. On the other hand, other 2 cases of the tumor from one follicle implant and consecutively transplanted spleens (2SPO-1 follicle) resulted in gonad stromal tumor.

Most of the remaining 17 cases of double im-

plantation of ovaries into spleens (DIO) and sequential transplantation of the spleens with tumor (SPO) had the same result. These results seem to suggest that if the amount of ovarian implants is increased, then dysgerminomas will develop more frequently.

As we reported before, the testicular or ovarian tumors that had grown are either in diffuse shape under the capsule of spleen, bulged out but covered by intact capsule, or pedunculated.¹ The gonadal- or sex cord- stromal tumor composed of variable round or polygonal cells, which had clear cytoplasm with distinct nuclei, was Sertoli or Leydig cell tumor (Fig. 2-a). Certain tumor still contained spermatocytes in their atrophic seminiferous tubular structures (Fig. 3-a).

Ovarian tumors were mainly composed of ovarian cell types (granulosa-stromal cell tumor), but occasionally contained a portion of testicular type (Sertoli-stromal cell tumor (Fig. 3-b). We could see several eosinophilic material called as Call-Exner bodies, which were surrounded by granulosa cells (Fig. 2-b).

Seminoma was observed to be yellow to white colored, firm, and smooth (or occasionally lobulated) on their surface. The microscopic findings showed a typical feature of multiple infiltrated

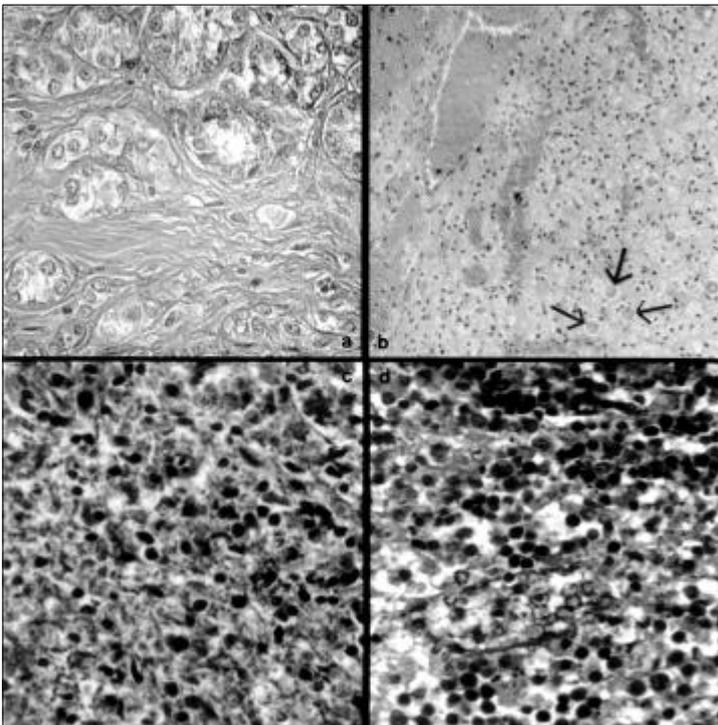


Fig. 2. Pathologies of tumors developed from the previously implanted gonads into spleen. a. Sertoli cell tumor, intermediated differentiated, 14 months after implant of one infantile testicle. Tubular and intersecting fibrous band structures are seen ($\times 400$). b. Lower magnification of granulosa cell tumor; 14 months after implant of one ovarian follicle. Multiple Call-Exner bodies (arrow) ($\times 200$). c & d. Seminoma, 27 months after implant of infantile testicle (c) and dysgerminoma, 15 months after double implant of each five follicles (d). Both tumors composed of a uniform population of large cells with clear cytoplasm and dense stained nuclei like primitive germ cells in cords or alveoli, and many lymphocyte infiltrations in the stroma ($\times 400$).

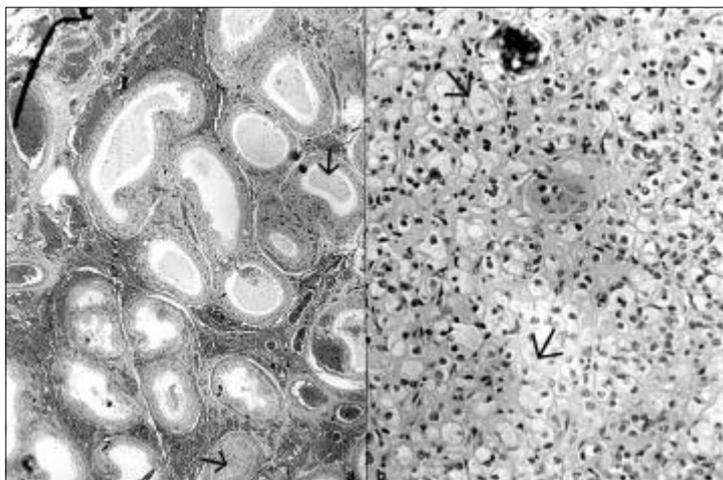


Fig. 3. a. Sertoli-Leydig cell tumor, still containing spermatocytes (small arrow) in their atrophic seminiferous tubular structures ($\times 200$). b. Granulosa-stromal cell tumor of ovary mixed with some clusters of clear cells of testicular type (big arrow) ($\times 400$).

lymphocytes and highly reactive immunoblast cells. Tumor cells were composed of a uniform population of large cells with clear cytoplasm and dense stained nuclei (Fig. 2-c).

Dysgerminomas were grossly solid, pink to brown colored mass with lobulated surface like a brain hemisphere. The size of our cases varied from 1.5 cm to 4.0 cm in diameter. The largest tumor reached up to 12 ± 9 cm in size. The microscopic features include largely rounded masses and polyhedral clear cells with large nuclei, like primitive germ cells in cords or alveoli. Many lymphocyte infiltrations were seen in the stroma (Fig. 2-d).

DISCUSSION

Since Biskind studied the development of tumor after the implantation of ovarian follicles into castrates' spleens in rodents, there were many studies done for tumor formation from ovary and testicle by implanting them into the spleen. Kojima A et al. reported the development of granulosa cell tumor from intrasplenic testicle of ACI rat with 46% of incidence rate.⁷ Nishida and Ueyama group injected adult or infantile testicle into the spleen, and observed higher incidence of tumor formation.⁸

Recently, intrasplenically implanted ovaries were reported to develop various gonadal stromal tumors at 77-80% incidence rate by Anisimov V. et al.⁹ Dysgerminoma was found only in young

1-month-old rats in their report, and age factor was considered as a mechanism of malignant tumor development. Ueyama group has observed these splenic implantations for 24 months, and none of the sex-cord stromal tumors were of germ cell origin.

Lipschultz enumerated the methods of experimental ovarian tumor formation, such as irradiation, applying carcinogens, ligation of ovarian vessels, ovarian transplantation, and prolonged administration of steroid.¹⁰ It was suggested that the hyper-stimulation of gonadotropin had an essential role in tumor development on the gonadal implant.⁴ Otsu I. et al. supported the mechanism of male gonad dysfunction in liver cirrhosis with a study on portocaval shunt in rat. They checked the level of the sex hormones in portocaval shunted rats, and assumed that the decreased metabolism of these hormones within the liver is caused by gonadal dysfunction via feedback mechanism of pituitary-gonad axis.¹¹

Now, two main factors are being speculated as the cause for the development of malignant tumors following gonad implant into the spleen of castrates: time (age) and hormone. Since certain portions of splenic gonad implants showed malignant transformation, such as seminoma or dysgerminoma, immunological and other factors seem to play a role in the pathogenesis. Age factor is now considered as an etiology for ovarian tumor, although dysgerminomas occur primarily in women under the age of 30.^{12,13} Long-term follow-up of this study revealed unusual tissue transformation

of malignant type tumor. We observed that seminoma developed from intrasplenic infantile testicle after long-term implantation via consecutive spleen transplantation.^{1,14}

For testicular tumors, Ueyama group did a followed-up study for as long as 24 months post-implant, which is a single biological life span for a rat. Not only did they observe Granulosa cell tumors, but they also found well-differentiated Sertoli and Leydig cell tumors as well as unclassified malignant cells. The gonadal- or sex cord-stromal tumor is a benign form of gonad tumor. The Sertoli or Leydig cell tumor, granulosa cells, theca cells, and their lutenized derivatives are included in this category. During the course of our observation of long-term testicular implant, many cases developed seminoma at far beyond rat's biologic life span of maximum 43 months.

We also found dysgerminoma, female equivalent of seminoma, in long-term follow-ups. Through consecutive spleen transplantation, the total duration of ovarian implantation were extended to 28.5-34 months. Moreover, we modified the implant mode in 11 cases by adding five follicles into the native or graft spleens already bearing ovarian tumors (after 1SPO and 2SPO) as shown in Table 1. All of these cases showed the same type of dysgerminoma. Further studies are required on molecular or immunological basis to determine which factor may have played a major role in the development of this kind of tumor.

However, considering the metabolism of gonadal hormones by the liver, the large amount of ovarian implant can cause the hormones to escape from the liver, ultimately affecting the pituitary-gonad axis. Theoretically the tumors would not grow in such case, but some have grown to a remarkable size of 9-12 cm in length.² Therefore, other mechanisms cannot be excluded as a possible etiology in our results. To elucidate the hormonal influence theory, the hormonal value in the portal vein and suprahepatic vena cava should be compared with one another.

In order to test this theory in other way, our laboratory tried to divert whole venous drainage from both ovaries by caval-portal shunt as previously described with 7 female rats.¹⁵ Moreover, we divided each oviduct to prevent possible collateral vein developments after diversion of

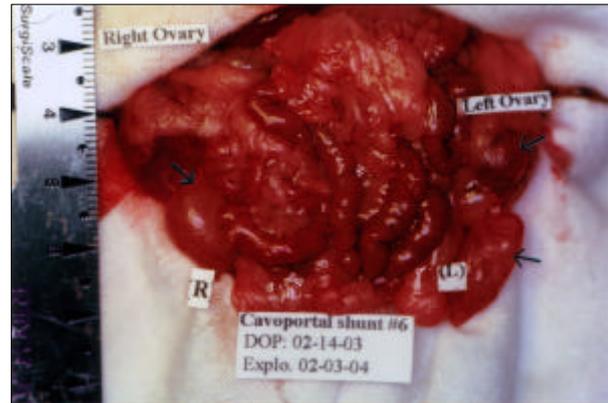


Fig. 4. Multiple ovarian cysts (arrow) developed from both enlarged ovaries 11.5 months after caval-portal shunt, the enlarged ovaries reached to 2.5-4 cm.

venous drainage. We expected all hormone secretion from both ovaries to be destroyed by the liver, and gonadotropic hormone to stimulate to develop certain ovarian tumor by feedback mechanism. However, after 11.5-12 months, the results were not satisfactory since lots of them showed enlargement with multiple cysts or with abscess of ovary in certain cases (Fig. 4). Through our observation it was apparent that hormonal theory alone could not be regarded as the sole cause of tumor growth.

There were certain gaps of 9 to 15 months, as shown between the first implant and each of the 1,2,3 SPT or 1,2,3 SPO. This is because the experimental protocol was designed to transplant spleens bearing gonads that were implanted for at least 9 to 15 months. When these spleen-transplanted rats survived for more than 9 to 15 months, we removed the spleens and sequentially transplanted them to other young rats to obtain tumor specimens at 9- to 15- and 18- to 25- (or 36-) month intervals.

The long-term follow-ups of the implanted gonads were made possible by applying the concept of the consecutive organ transplantation already proposed before to these studies.¹⁶ This could be used as an animal research model of gonad tumor development in future studies.

CONCLUSION

We presented the results after transplanting the

spleens with variable tumors. These tumors were induced by implanting gonads into the donor spleens. For the gonad implants, we used one intact infantile testicle including epididymis in male and one, five, or ten mature ovarian follicles in female rats. All cases were observed over long-term basis via consecutive spleen transplantation; up to maximum of 43 months in testicle and up to maximum of 34 months in ovary. Most testicular tumors with less than 26 months of implant-duration showed benign gonad stromal tumor. Most ovarian tumors developed from one-follicle implant, even with longer implant-duration, also showed benign gonadal stromal tumor. While the implanted infantile testicles tended to transform to seminoma over longer periods of time, most cases of ovarian implantation with increased amount of follicles showed dysgerminoma instead in similar duration. The different pathological results on the long-term observation of the testicle implants and the increased amount of ovarian follicles suggest that other mechanisms could be the cause of malignant tumor transformation in each gonad. However, further studies are required to clarify their pathogenesis.

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