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Original contribution

Allelotyping analysis suggesting a consecutive progression from intratubular germ cell neoplasia to seminoma and then to embryonal carcinoma of the adult testis☆,☆☆

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Summary Among adult testicular germ cell tumors, the pathogenesis of embryonal carcinoma remains a matter of debate. Some studies suggest a single consecutive progression from intratubular germ cell neoplasia, unclassified (IGCNU), to seminoma and then to embryonal carcinoma; others suggest that seminoma and embryonal carcinoma derive independently from IGCNU. This allelotyping study aimed to clarify the genetic relationship between embryonal carcinoma components and coexisting seminoma and/or IGCNU components. From a cohort of 18 patients with embryonal carcinoma, 11 coexisting seminoma components and 14 coexisting IGCNUs were identified. DNA isolated from each laser-microdissected tissue was subjected to polymerase chain reaction and loss of heterozygosity (LOH) analysis, using 20 polymorphic markers located on 12 chromosome arms (3q, 5q, 6p, 9p, 10q, 11p, 12p, 12q, 13q, 17p, 17q, and 18q). The concordance rate for allelic patterns was 82% between IGCNU and the coexisting seminoma components, 71% between IGCNU and the coexisting embryonal carcinoma components, and 80% between seminoma components and the coexisting embryonal carcinoma components. Estimation of probability indicated that these events were very unlikely to have occurred by chance. The total frequency of LOH increased progressively from IGCNU to seminoma and then to embryonal carcinoma, with statistically significant differences. In 7 cases with 3 histologic components, 28 chromosomal loci that showed LOH in the seminoma and embryonal carcinoma components were identified, and 15 (54%) retained heterozygosity in the coexisting IGCNUs. These findings suggest that a consecutive progression from IGCNU to seminoma, and ultimately, to embryonal carcinoma mainly occurred in the testicular germ cell tumor cases.

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1. Introduction

Adult testicular germ cell tumor (TGCT) is the most frequent malignant solid tumor among men aged 15 to 45 years, and its incidence has been increasing over the past 30 years [1]. Clinicopathologically, TGCTs are divided into 2 entities: seminomas and nonseminomatous germ cell tumors (NSGCTs). Embryonal carcinoma is the most frequent histology observed in NSGCTs [1]. Despite advances in the management of TGCT, a small group of patients with late relapse or tumors refractory to chemotherapy show poor prognosis [2,3]. NSGCTs are more likely to be metastatic at presentation, and those in advanced stages confer worse prognosis than seminomas at an equivalent stage of disease. In addition, the predominance of embryonal carcinoma components in a clinical stage I NSGCT warrants additional therapy after orchiectomy because it is commonly associated with a high risk of failure on surveillance alone [4-6]. This poor prognosis is also attributed to the fact that little is known about the etiology and progression of embryonal carcinoma.

Although intratubular germ cell neoplasia, unclassified (IGCNU), is generally accepted as a form of a noninvasive precursor lesion for seminoma and embryonal carcinoma [7,8], the developmental relationship between seminoma and embryonal carcinoma remains poorly understood. Some studies suggest a single consecutive progression from IGCNU to seminoma and then to embryonal carcinoma ("linear" progression), whereas others suggest that seminoma and embryonal carcinoma independently derive from IGCNU ("nonlinear" progression). The former theory is supported by morphologic examination [9], DNA flow cytometry [10], and cytogenetic analyses [11,12]. Recent findings using analysis of global DNA methylation [13] and immunohistochemical expression of stem cell markers [14] support the latter theory as well as the former one.

Inactivation of tumor suppressor genes plays a central role in the development and progression of many human cancers and is generally identified by loss of heterozygosity (LOH) typing at polymorphic chromosomal loci. Several studies have reported that TGCTs frequently showed allelic losses in 2p, 3p, 3q, 5p, 9p, 9q, 10q, 11p, 12q, 17p, 17q, 18p, 18q, and 20p, suggesting a role for the inactivation of several candidate tumor suppressor genes in the development of TGCTs [15-23]. Using allelotyping analysis, previous studies have reported a clonal relationship between the histologic components of mixed TGCTs (ie, tumors comprising $\geq$2 histologic components). Rothe et al [24] analyzed 20 mixed TGCTs for LOH, and of these tumors, 11 (55%) showed similar LOH patterns between various histologic components. Faulkner et al [25] performed allelotype analysis of 87 TGCTs, including 2 mixed tumors with IGCNU, seminoma, and embryonal carcinoma components, and a similar LOH pattern was noted in all 3 components of these mixed tumors. However, these previous studies were designed for mainly pure-type invasive tumors or embryonal carcinomas without seminoma components, and they included only a few tumors with both seminoma and embryonal carcinoma components. To our knowledge, no previous study has examined the clonal relationships between IGCNU, seminoma, and embryonal carcinoma components and has discussed the progression pathway (ie, whether linear or nonlinear progression) in detail.

In this study, we extracted DNA from tumor cells of the IGCNU, seminoma, and embryonal carcinoma components of 18 TGCTs using laser microdissection. These cases included embryonal carcinoma components with seminoma and/or IGCNU components, which represented the histologic continuum. Then, we performed polymerase chain reaction (PCR)–based LOH analyses using 20 polymorphic markers located on 12 chromosomal arms, on which relatively frequent allelic losses have been reported in TGCTs [15-25]. We compared allelic statuses among the histologic components of TGCTs. The aims of this study were to clarify the progression pathway of embryonal carcinoma and to identify the chromosome loci demonstrating LOH that play a role in its progression.

2. Materials and methods

2.1. Patients enrolled and histologic components analyzed

According to the World Health Organization criteria [1], a total of 18 patients with TGCTs, which had embryonal carcinoma components with IGCNU and/or seminoma components, were identified on reviewing files of the Department of Laboratory Medicine, National Defense Medical College Hospital, Tokorozawa, Japan. All these patients underwent primary surgery between 1988 and 2008, and none had undergone preoperative chemotherapies or radiation therapies. Clinicopathologic characteristics of the analyzed cases are shown in Table 1. Clinical staging of disease was performed according to the International Union Against Cancer System [26]. From this cohort of 18 patients with embryonal carcinoma, a total of 25 coexisting histologic components, 11 seminomas and 14 IGCNUs, were identified. Of the 18 patients, 7 (cases 1-7; case category a) had all 3 histologic components (IGCNU, seminoma, and embryonal carcinoma), and 11 patients had 2 components (seminoma and embryonal carcinoma in 4 cases [cases 8-11; case category b] and IGCNU and embryonal carcinoma in 7 cases [cases 12-18; case category c]). Although we made at least 10 blocks for each case and evaluated all slides, IGCNU was not observed or not enough for allelotyping analysis in 4 cases. In cases 9 and 11, no seminiferous tubule was observed because a large tumor occupied almost all testicular tissue. In cases 8 and 10, we detected only a tiny volume of IGCNU, which was not enough for the analysis. Ten cases (cases 1-3, 7-9, 12, 13, 15, and 16) included 1 or