J. Comp. Path. 2016, Vol. 155, 5–14

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

www.elsevier.com/locate/jcpa

NEOPLASTIC DISEASE

Spermatogonial Nature of the Germ Cell Component of Canine Testicular Mixed Germ Cell–Sex Cord Stromal Tumours

S. Mizukami[*,†], T. Murakami[‡], T. Tanaka[*,†], N. Machida[§], K. Nomura[¶], T. Yoshida[*] and M. Shibutani[*]

** Laboratory of Veterinary Pathology, [‡] Laboratory of Veterinary Toxicology, [§] Laboratory of Veterinary Clinical Oncology, Tokyo University of Agriculture and Technology, Tokyo, [†] Pathogenetic Veterinary Science, United Graduate School of Veterinary Sciences, Gifu University, Gifu and [¶] Veterinary Diagnostic Laboratory, Marupi Lifetech Co. Ltd., Osaka, Japan*

Summary

The present study has characterized the germ cell component of canine testicular mixed germ cell–sex cord stromal tumours (MGSCTs) by examining the histological nature and histochemical and immunohistochemical features using gonocytic and spermatogonial cellular markers, c-Kit, placental alkaline phosphatase (PLAP), protein gene product 9.5 (PGP9.5), Sal-like protein 4 (SALL4), and the periodic acid–Schiff (PAS) reaction. Histologically, all 45 examples of MGSCTs were classified as spermatocytic seminomas (SSs) and Sertoli cell tumours in combination. The germ cell component of all MGSCTs was negative by PAS staining. Immunohistochemically, PLAP immunoreactivity was lacking in the germ cell component of all MGSCTs, which is not consistent with a gonocytic origin. The germ cell component was positive for PGP9.5 and SALL4 in all MGSCTs and positive for c-Kit in 53% of MGSCTs, which is consistent with the phenotype of spermatogonia. Furthermore, the germ cell component in 71% of MGSCTs had moderate immunoreactivity for SALL4, which is suggestive of a spermatogonial phenotype. Conversely, 29% of cases had a minor population of germ cells showing strong SALL4 immunoreactivity, suggesting a phenotype similar to prespermatogonia. The results suggest that the germ cell component of canine MGSCTs is morphologically classified as SS, with the majority of cases showing the spermatogonial phenotype and some cases containing a small population of prespermatogonia.

Keywords: dog; mixed germ cell–sex cord stromal tumour; protein gene product 9.5; Sal-like protein 4

Introduction

Testicular mixed germ cell–sex cord stromal tumours (MGSCTs) are very rare in adult men (Michal *et al.*, 2006). Histologically, they consist of germ cells and sex cord stromal cells intimately admixed with each other (Talerman, 1980). The morphology of germ cells in human testicular MGSCTs is similar to that observed in spermatocytic seminomas (SSs), with a variation in the cell size from small and deeply basophilic to large and blastic (Michal *et al.*, 2006), in contrast with the monotonous proliferation of gonocytic cells in classical seminomas (SEs; Eble *et al.*, 2004). Conversely, MGSCTs are relatively frequently encountered testicular tumours in older dogs, in association with cryptorchidism (Patnaik and Mostofi, 1993; Bush *et al.*, 2011).

In men, testicular gonocytes can be identified by the periodic acid–Schiff (PAS) reaction, which detects glycogen (Müller *et al.*, 1987; Mostoȼ and Sesterhenn, 1998), and immunohistochemically by labelling for placental alkaline phosphatase (PLAP)

Correspondence to: M. Shibutani (e-mail: mshibuta@cc.tuat.ac.jp).

0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2016.04.002

and c-Kit. PLAP is a membrane-bound glycosylated enzyme expressed primarily in the placenta. Expression of PLAP in testicular germ cells can be recognized until the pre-spermatogonial stage (Mitchell *et al.*, 2014). c-Kit is a receptor tyrosine kinase that is critical for the activation and growth of various cell types including haemopoietic stem cells, mast cells, melanocytes and germ cells (Gommerman *et al.*, 1997). It is expressed throughout the postpubertal stage of spermatogonia in mice and rats (Yoshinaga *et al.*, 1991; Dym *et al.*, 1995). Because neoplastic cells of human SEs express PLAP and c-Kit, and are PAS positive, human SE has been thought to originate from gonocytes (Stoop *et al.*, 2008). In contrast, SSs, which are clinically benign, rare tumours derived from more differentiated cells, lack expression of PLAP and c-Kit, and are PAS negative in men (Dekker *et al.*, 1992; Cummings *et al.*, 1994). Moreover, Sal-like protein 4 (SALL4), a stem cell marker of broad cellular origin, is expressed in human testicular and ovarian germ cell tumours (Cao *et al.*, 2009). In human seminomas, SEs have shown strong nuclear SALL4 expression, while SSs have weak to moderate labelling intensities (Cao *et al.*, 2009).

There are reports showing that all canine seminomas are morphologically diagnosed as SS, of which 82–96% are negative for PLAP and 27–60% are negative for c-Kit (Bush *et al.*, 2011; Thorvaldsen *et al.*, 2012). These findings suggest that PLAP may be better than c-Kit for differential diagnosis between SE and SS in dogs. We have recently reported that seminomas characterized morphologically as SS are 100% negative for PLAP and PAS reactivity, while most cases diagnosed morphologically as SE are also negative for PLAP and PAS reactivity (Hara *et al.*, 2014). In terms of c-Kit immunoreactivity, only half of morphologically diagnosed SEs show positive immunoreactivity, which is in contrast with the c-Kit-negative immunoreactivity of most morphologically-diagnosed SSs (Hara *et al.*, 2014). These results suggest that negativity for all these markers may support the SS nature of canine seminomas. However, all of these markers do not correspond to all morphologically-diagnosed SEs. We have proposed that this is because canine SS may be derived from spermatogonia, which can differentiate into spermatocytes, and that many canine SEs may consist of neoplastic cells that have lost their gonocytic character. Conversely, protein gene product 9.5 (PGP9.5), a ubiquitin carboxyl-terminal hydrolase, is expressed in germ cells and neoplastic germ cells of MGSCTs in dogs (Owston and Ramos-Vara, 2007). While there have been no reported studies of human gonadal tumours, we have recently reported diffuse PGP9.5 immunoreactivity in two canine dysgerminomas and all examined SEs and SSs (Hara *et al.*, 2014). Moreover, we have shown that all SEs and SSs exhibit diffuse nuclear SALL4 immunoreactivity in dogs. However, more SEs show strong immunoreactivity than SSs (Hara *et al.*, 2014).

In human testicular tumours, there is only one case report that examined the immunohistochemical characteristics of neoplastic germ cells in MGSCTs. In that report, neoplastic germ cells of three MGSCTs were negative for PLAP and c-Kit immunoreactivity, suggestive of SS characteristics (Michal *et al.*, 2006). Some canine MGSCTs exhibit positive immunoreactivity for both c-Kit and PLAP (Hohšteter *et al.*, 2014), and a positive PAS reaction (Banco *et al.*, 2015), suggestive of SEs, while a minor population of MGSCTs are negative for all germ cell markers, suggestive of SSs (Banco *et al.*, 2015). These results imply that there may be canine MGSCTs with a germ cell component consisting of SE, which is different from the germ cell component consisting of SS in human counterparts. However, it remains unclear whether these immunohistochemical and histochemical features of the germ cell component in canine cases are in accordance with the histological features of SE and SS.

The present study was conducted in order to investigate differentiation of the germ cell component in canine MGSCTs by comparing the histological nature and histochemical and immunohistochemical features of these tumours.

Materials and Methods

We examined 45 cases of MGSCTs obtained from the archives of Marupi Lifetech Co., Ltd., Osaka, Japan, collected during 2007 and 2011. Histopathological diagnosis was based on assessment by light microscopy and haematoxylin and eosin (HE) staining according to the World Health Organization (WHO) histological classification system for tumours of domestic animals (Kennedy *et al.*, 1998). Germ cell populations were classified into SE or SS in accordance with the WHO/International Agency for Research on Cancer classification of testicular tumours in man (Eble *et al.*, 2004). In addition, proliferation patterns were further classified into intratubular, diffuse or intratubular/diffuse (Grieco *et al.*, 2007).

Tumour tissues were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (3 μm) were subjected to HE and PAS staining. Immunohistochemistry (IHC) was performed using the avidin–biotin–peroxidase complex technique, using the Vectastain® Elite ABC Kit (Vector Laboratories Inc., Burlingame, California, USA) and primary antibodies against c-Kit,