J. Comp. Path. 2017, Vol. ■, 1–5

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

www.elsevier.com/locate/jcpa

NEOPLASTIC DISEASE

Immunohistochemical Expression of Placental Alkaline Phosphatase in Five Cases of Seminoma in Rabbits

B. Banco*, J. Ferreira da Silva†, S. Cotti Cometti*, D. Stefanello* and V. Grieco*

** Dipartimento di Medicina Veterinaria, Università degli Studi di Milano, Milan, Italy and † Faculdade de Medicina Veterinária, Avenida da Universidade Técnica, Lisbon, Portugal*

Summary

Testicular seminoma is reported in the rabbit but data about the immunophenotype of these tumours are lacking. The classification of human testicular germ cell tumours includes spermatocytic tumour (ST) originating from the post-pubertal spermatogonia/spermatocytes, which metastasizes rarely, and seminoma (SE), originating from gonocytes, which is malignant and metastasizes frequently. Gonocytes express placental alkaline phosphatase (PLAP) and are stained with periodic acid–Schiff (PAS). We report five cases of seminoma in pet rabbits. Microscopically, all the cases were diffuse seminoma and in one case there was metastasis to a sublumbar lymph node. Immunohistochemical expression of PLAP was diffuse in this metastatic tumour, in two other cases it was multifocal, in another it was limited to rare cells and in the remaining case was negative. PAS-positive cells were detected only in the four cases that expressed PLAP. These four cases were therefore classified as SE and the tumour without PLAP labelling or PAS staining was defined as ST. Both forms of human germ cell tumour therefore occur in the rabbit. SE appears to be well represented and may show metastasis, paralleling the human counterpart. The results of this study provide a basis for further evaluations of the rabbit as a possible animal model for the study of human SE.

Keywords: immunohistochemistry; placental alkaline phosphatase; rabbit; seminoma

Spontaneously arising testicular tumours are reported rarely in rabbits. Among these tumours, Leydig cell adenoma (interstitial cell tumour) and seminomas are the most frequently reported, while teratoma, gonadoblastoma and granular cell tumour are rare (Meier *et al.*, 1970; Flatt and Weisbroth, 1974; Irizarry-Rovira *et al.*, 2008; Suzuki *et al.*, 2011). The first case of a spontaneously arising seminoma in a rabbit was described in 1918 by Paine and Peyron in an adult laboratory rabbit. Other cases were recorded more recently, occurring simultaneously with an interstitial cell tumour (Roccabianca *et al.*, 1999; Veeramachaneni and Vandewoude, 1999) or as a unilateral/bilateral mass (Brown and Stafford, 1989; Anderson *et al.*, 1990). Finally, a case of metastasizing rabbit testicular seminoma was described in 2012 (Banco *et al.*, 2012).

In man, seminomas are common and account for approximately one half of all testicular germ cell tumours (TGCTs) (Bosl and Motzer, 1997). In the most recent WHO classification of human TGCTs, spermatocytic tumour (ST), formerly named 'spermatocytic seminoma', and seminoma (SE), formerly named 'classical seminoma', are included (Moch *et al.*, 2016). ST is considered to be a rare tumour and represents a more differentiated type of TGCT, originating from post-pubertal spermatocytes or

Correspondence to: B. Banco (e-mail: barbara.banco@unimi.it).

0021-9975/$ - see front matter

http://dx.doi.org/10.1016/j.jcpa.2017.01.008

Please cite this article in press as: Banco B, et al., Immunohistochemical Expression of Placental Alkaline Phosphatase in Five Cases of Seminoma in Rabbits, Journal of Comparative Pathology (2017), http://dx.doi.org/10.1016/j.jcpa.2017.01.008

spermatogonia (Looijenga *et al.*, 2007). ST typically occurs in men aged over 50 years and usually has a self-limiting, benign behaviour (Aggarwal and Parwani, 2009). Conversely, SE is the most common TGCT, is malignant, frequently metastasizing and occurs typically in young adults (Looijenga *et al.*, 2007). SE derives from gonocytes, undifferentiated seminal progenitor cells that in the human fetal testis may be found during the first trimester of pregnancy, subsequently developing into prespermatogonia and spermatogonia (Gaskell *et al.*, 2004). Gonocytes can be recognized immunohistochemically by the expression of placental alkaline phosphatase (PLAP) and histochemically by the periodic acid–Schiff (PAS) reaction, due to the presence of glycogen (Moch *et al.*, 2016). ST, derived from more differentiated cells, does not express PLAP and is always PAS negative (Cummings *et al.*, 1994).

In rabbits, seminoma and the precursor lesion, carcinoma in situ (CIS), have been suggested to have morphological similarities to the human counterparts (Veeramachaneni and Vandewoude, 1999). The aim of the present study was to further characterize seminoma in the rabbit in light of the human classification.

Paraffin wax blocks of formalin-fixed testicular seminomas from five pet rabbits (ranging in age from 5 to 9 years) were selected from the departmental archives. Four cases had been obtained after castration and were submitted for diagnostic purposes. For two of these four cases, paraffin wax blocks of both testes were available. The fifth case, from a rabbit that was humanely destroyed intraoperatively, was a testicular seminoma affecting the left testis and metastasizing to the sublumbar abdominal lymph node. This case has been the subject of a separate report (Banco *et al.*, 2012).

Serial sections (5 μm) were taken from each block. Two sections were stained with haematoxylin and eosin (HE) and PAS and others were subjected to immunohistochemistry (IHC) with a commercial avidin–biotin–peroxidase complex (ABC) kit (Vectastain Standard Elite; Vector Laboratories, Burlingame, California, USA). For IHC, sections were dewaxed, treated with H_2O_2 0.3% in methanol for 20 min and rehydrated. After a heat-induced epitope unmasking procedure (10 min in pH 8.0 EDTA buffer in a 600 W microwave oven; Thermo Fisher Scientific, Waltham, Massachusetts, USA), sections were blocked for 20 min with normal horse serum at room temperature and then incubated with monoclonal mouse anti-human PLAP antibody (Clone 8A9, Agilent Technologies Dako, Glostrup, Denmark) diluted in pH 7.5 Tris buffer (1 in 25), and incubated at 4°C overnight. After washing in Tris buffer, the sections were incubated with biotinylated anti-mouse IgG antibody (diluted 1 in 200) at room temperature for 30 min. After washing, the peroxidase-conjugated ABC (diluted 1 in 100) was allowed to react at room temperature for 30 min. The immunohistochemical reaction was developed with 3-amino-9-ethylcarbazole (AEC) (Vector Laboratories) for 10 min following the manufacturers' instructions. Sections were counterstained with Mayer's haematoxylin. Internal positive controls consisted of the myoid peritubular cells and the smooth muscle cells of vessel walls. For a negative control, the primary antibody was replaced by normal horse serum.

On the basis of histological pattern, the seminomas were classified as intratubular or diffuse, according to the WHO classification system for tumours of domestic animals (Kennedy *et al.*, 1998). The mitotic count was assessed by counting the mitotic figures present in 10 high-power fields (HPFs, ×400). The range and mean value for each case were recorded. A summary of the histological and immunohistochemical findings is given in Supplementary Table 1.

Histologically, in all cases, more than 80% of the testicular parenchyma was replaced by a poorly demarcated, densely cellular tumour, composed of round cells separated by scant fibrovascular stroma. Neoplastic cells, 15–20 μm in diameter, had distinct cell borders, a high nuclear:cytoplasmic ratio, moderate amount of eosinophilic cytoplasm, and large, round, centrally located nuclei with vesicular chromatin and 1 or 2 prominent, centrally located, nucleoli (Fig. 1). Anisokaryosis and anisocytosis were marked, and mitotic figures ranged from 0 to

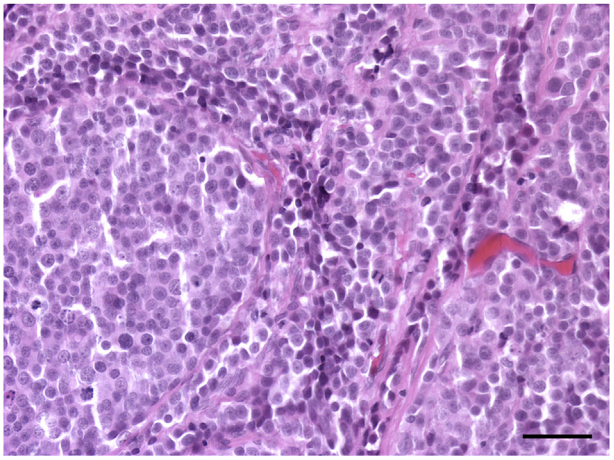

Fig. 1. Rabbit testis, seminoma. The testicular parenchyma is replaced by a densely cellular neoplasm, composed of sheets of round cells separated by scant fibrovascular stroma. Lymphocytic aggregates between the neoplastic cells are present. HE. Bar, 60 μm.

Please cite this article in press as: Banco B, et al., Immunohistochemical Expression of Placental Alkaline Phosphatase in Five Cases of Seminoma in Rabbits, Journal of Comparative Pathology (2017), http://dx.doi.org/10.1016/j.jcpa.2017.01.008