



## CASE REPORT

### Nephroblastoma with Mucus-Producing and Argentaffin Cells in a Pig

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#### ABSTRACT

Nephroblastoma is the most common primary renal neoplasm of swine and man. This report describes a swine nephroblastoma localized at the caudal pole of the left kidney, extending to the renal hilum which, on light microscopy, showed unusual mucus-producing epithelial cells of many tubules. The presence of intracytoplasmic mucin was confirmed by PAS-diacetate positivity and alcianophilia both at pH 2.5 and at 1.0. Mucin-producing cells revealed cytokeratin 19 and pankeratin AE1/AE3 positivity, and some of them a chromogranin A immunohistochemical positivity; they were also positive for Grimelius stain. In veterinary medicine, even if the occurrence of mucus-producing cells in nephroblastoma has rarely been observed, this is the first description of a case of swine nephroblastoma with mucus-producing cells positive for neuroendocrine markers, thus a neuroendocrine origin was suspected.

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#### INTRODUCTION

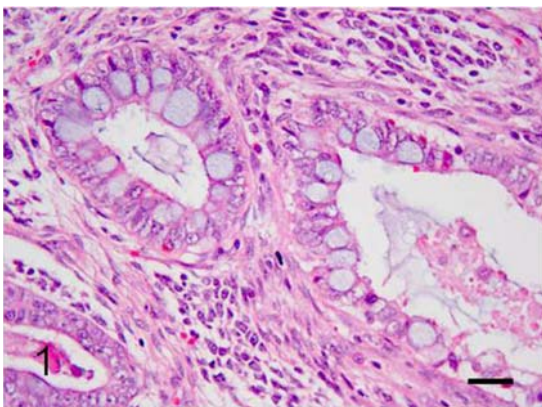
Nephroblastoma or Wilms' tumor is a complex embryonic renal neoplasm in which the tissue of origin is the metanephric blastema and that the stromal cells and blastema develop from a common stem cell. The tumor is a mixture of embryonic renal tissue with immature glomerular-like buds, tubules, and myxomatous mesenchyme in various amounts (Meuten, 2002). Swine nephroblastomas are classified on the basis of their contents of mesenchymal and epithelial component in: nephroblastic only, nephroblastic predominance, epithelial only and epithelial predominance, and nephroblastic equal to epithelial (Hayashi *et al.*, 1986). Although swine nephroblastoma is the most common renal neoplasm in the pig (Meuten, 2002; Grieco *et al.*, 2006) there was no insights of the presence of mucus-producing and argentaffin cells in this species.

**Case history and methodology:** The case described herein was observed during post-mortem examination in a swine slaughter-house. The pig was an Italian heavy pig (live weight of between 150-170 kg) aged 9 months. At clinical examination the pig showed good state of nutrition and health. At inspection the pig presented a large tumour (12 x 8 x 10 cm) at the caudal pole of the left kidney; no macroscopical evidence of metastases was found. The mass

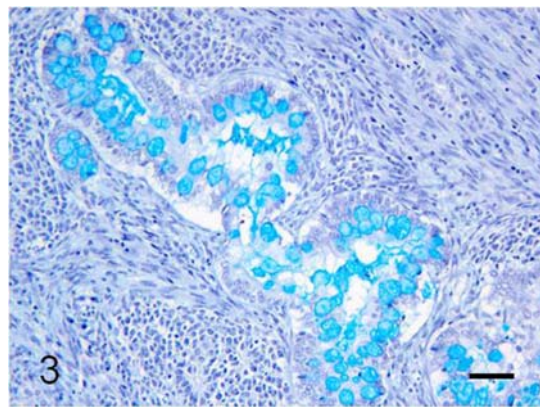
was roundish, whitish and well-defined, surrounded by a thin capsule compressing the adjacent renal parenchyma and extending to the renal hilum. On the cut surface it was firm and divided into lobules by connective tissue septa;

Tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin, sectioned at 4 µm and stained with hematoxylin and eosin (H&E). Subsequently, Periodic Acid-Schiff (PAS), PAS-diacetate and alcian blue pH 2.5 and pH 1.0 methods, and Grimelius were performed.

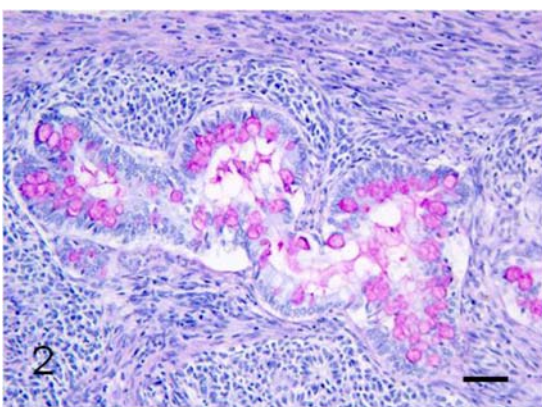
To identify the phenotype of the mucin-producing cells, an immunohistochemical study was also carried out using cytokeratin 19 (clone BA 17, 1:40), pankeratin (clone AE1/AE3, 1:50), anti-smooth muscle  $\alpha$ -actin (clone 1 A4, 1:100), anti-vimentin (clone V9, 1:40), and polyclonal antibody against chromogranin A (1:100) to confirm the presence of cytoplasmic argyrophilic granules; all antibodies were from DAKO. The sections were dewaxed in toluene and rehydrated. Endogenous peroxidase was blocked by immersion in methanol containing 0.3% hydrogen peroxide for 30 min. Sections were then rinsed in Tris buffer, immersed in citrate buffer (2.1 g citric acid monohydrate/liter distilled water), pH 6.0, incubated for two 5 min periods in a microwave oven at 750 W, and allowed to cool at room temperature (approximately 20 min). The primary antibodies were applied overnight at 4°C and were followed by a commercial streptavidin-biotin-peroxidase kit (LSAB kit, DAKO, Amsterdam), and diaminobenzidine as



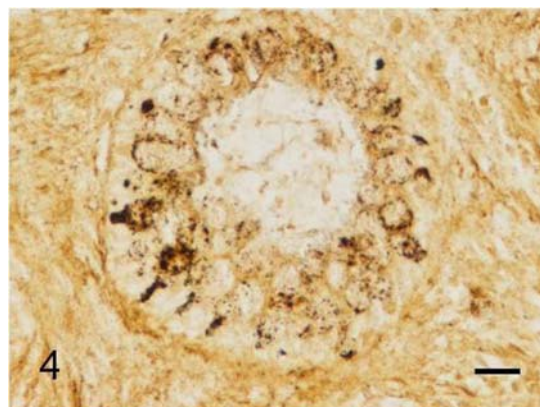
**Fig. 1:** Photomicrograph of nephroblastomas in kidney of a pig showing tubular epithelium of tubules with mucus-producing cells having abundant intracytoplasmic mucin content and eccentric nuclei. H&E stain. Bar=80µm.



**Fig. 3:** Photomicrograph of nephroblastomas in kidney of a pig showing the presence of acid mucopolysaccharide content (sulphurated type) confirmed by alcian blue pH 1.0 positivity. Bar = 160µm.



**Fig. 2:** Photomicrograph of nephroblastomas in kidney of a pig showing the presence of intracytoplasmic mucin (neutral mucopolysaccharide content) confirmed by PAS-diastrase positivity. Bar = 160µm.

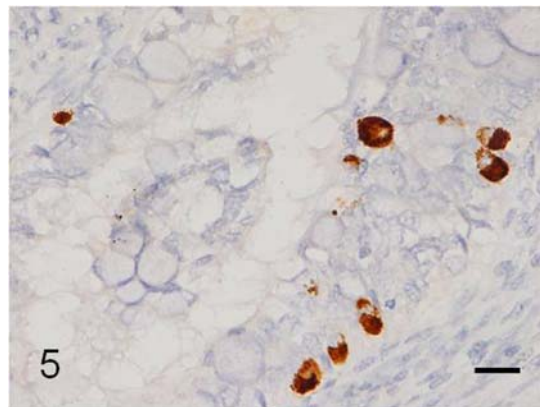


**Fig. 4:** Photomicrograph of nephroblastomas in kidney of a pig showing some mucus-producing cells positive to Grimelius stain, the argyrophilic granules are dark. Bar = 80 µm.

the chromogen (0.04% for 7 min). The sections were then counterstained with Papanicolaou hematoxylin, rinsed in tap water, dehydrated, and coverslipped. For negative controls, an unrelated monoclonal antibody of the same isotype and similar protein concentration was used instead of the primary monoclonal antibody.

## RESULTS

On microscopic examination, a lobulated and well demarcated, encapsulated expansile neoplasm composed of different cell populations (epithelial, mesenchymal and blastematos cells) was present. The epithelial component was predominant and composed of variably sized and shaped tubular structures and glomeruloid bodies. The tubules were lined by a single to several layers of epithelial cells that often showed mucus-producing cells having abundant intracytoplasmic mucin content and eccentric nuclei (Fig. 1); glomeruloid bodies consisted of tufts of cells surrounded by a lumen lined by epithelial cells. The mesenchymal component organized in bundles and septa dividing the tumour in many lobules, was composed of stellate to spindle fibroblasts, with indistinct cell borders, moderate amount of eosinophilic cytoplasm, and oval to elongated nucleus with dispersed chromatin. Smooth and striated muscle fibres were present. A blastemal component was also present, consisting of



**Fig. 5:** Photomicrograph of nephroblastomas in kidney of a pig showing some mucus-producing cells positive to chromogranin A. Bar = 80µm.

polygonal cells arranged in densely cellular aggregates, with indistinct cell borders, eosinophilic cytoplasm, round to oval nuclei with clumped chromatin and 1 to 3 nucleoli. The mitotic rate for all the cell components was less than 1 for high power field (400x). Multifocally in the tumoral stroma there were scattered lymphocytes.

The presence of intracytoplasmic mucin in the epithelial cells of the tubules was confirmed by PAS and PAS-diastrase (Fig. 2) positivity and alcianophilia both at pH 2.5 and at 1.0 (Fig. 3). Some of these cells were also

positive for Grimelius stain containing cytoplasmic argyrophilic granules, (Fig. 4).

In the present case the cytoplasm of tubular epithelial cells producing mucus were all positive to cytokeratin 19 and pankeratin and around 20% to chromogranin A (Fig. 5). They were negative to  $\alpha$ -actin and vimentin. The other tubules without mucin presence were almost all positive for pankeratin, and negative to cytokeratin 19,  $\alpha$ -actin, vimentin and chromogranin A. The glomeruloid-like structure and nephroblastic cells were negative to cytokeratin 19, pankeratin,  $\alpha$ -actin, vimentin and chromogranin A. The stromal fibroblasts were positive for  $\alpha$ -actin and vimentin. Mesenchymal blastema was only vimentin positive.

### DISCUSSION

In human medicine, nephroblastomas with descriptions of mucus-producing epithelial structures are rarely reported (Min and Seo, 1994; Sultan *et al.*, 2010). More common in nephroblastoma is the secretion of mucin in the sera, urine and in cell-free extract, although at histological examinations no mucus-producing cells are present (Miller *et al.*, 1990).

The presence of mucin production in nephroblastoma has already been reported in veterinary medicine; Nielsen and Moulton (1990) describe “*epithelial structures forming cysts or containing mucus-secreting cells or forming nests of squamous epithelium*”. More recent descriptions of nephroblastoma in domestic animals outline that in a minority of cases “*there are cystic structures lined by cuboidal epithelium or squamous epithelium with or without the presence of mucus, sloughed epithelial cells, or keratin*”; however, the species of occurrence was not specified and further histochemical/immunohistochemical characterization of these mucus-producing cells was not pursued (Meuten, 2002). The immunohistochemical features of this case showed that mucus-producing cells had an epithelial phenotype being positive to cytokeratin 19 and pankeratin, and negative to vimentin and  $\alpha$ -actin. These mucin-containing cells revealed a neutral and acid mucopolysaccharide content (these latter both carboxylated and sulphorated) confirmed by positivity to PAS-diastase and Alcian blue (pH 2.5 and 1.0), respectively. Moreover, some of these cells were positive to Grimelius stain and chromogranin A indicating the presence of neuroendocrine granules.

In human medicine, neuroendocrine differentiation in Wilms’ tumor was reported and neuroendocrine granules could be identified in the tubules along with mucus-producing cells (Min and Seo, 1994). Neuroendocrine granules could also be found in blastematos areas as described by some Authors (Min and Seo, 1994). These cells are probably APUD (Amine Precursors Uptake and decarboxylation) derived cells with both mucinous and enterochromaffin granules, as described in other tumors such as in goblet cell carcinoids (Roy and Chatty, 2010).

In human and veterinary medicine, neuroendocrine differentiation associated with mucin-production by neoplastic cells has been already observed in other tumours (Brunetti *et al.*, 2003; Roy and Chatty, 2010; Bartley *et al.*, 2011) but it is the first time that mucin-producing epithelial cells with neuroendocrine differentiation are described in a porcine nephroblastoma.

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