Aging and Senescence in Canine Testes

Veterinary Pathology
2019, Vol. 56(5) 715-724

DOI: 10.1177/0300985819843683
journals.sagepub.com/home/vet

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Abstract

Senescent cells accumulate with age but tissue-based studies of senescent cells are limited to selected organs from humans, mice, and primates. Cell culture and xenograft studies have indicated that senescent cells in the microenvironment may play a role in tumor proliferation via paracrine activities. Dogs develop age-related conditions, including in the testis, but cellular senescence has not been confirmed. We hypothesized that senescent cells accumulate with age in canine testes and in the microenvironment of testicular tumors. We tested the expression of the established senescence markers γH2AX and p21 on normal formalin-fixed, paraffin-embedded testes from 15 young dogs (<18 months of age) and 15 old dogs (7–15 years of age) and correlated the findings with age-dependent morphological changes. A statistically significant age-dependent increase in the percentage of p21-expressing cells was observed for testicular fibroblasts (4-fold) and Leydig cells (8-fold). However, p21-expressing cells were still a rare event. In contrast, the percentage of γH2AX-positive cells did not increase with age. P21- and γH2AX-expressing cells were rare in the microenvironments of tumors. Age-dependent morphological changes included an increased mean number of Leydig cells per intertubular triangle (2.95-fold) and a decreased spermatogenesis score. To our surprise, no age-related changes were recorded for interstitial collagen content, mean tubular diameter, and epithelial area. Opposed to our expectations based on previous in vitro data, we did not identify evidence of a correlation between age-associated accumulation of senescent cells and testicular tumor development. Understanding the role of the microenvironment in senescence obviously remains a challenging task.

Introduction:

Cellular senescence **is defined as**…

Current knowledge on senescence has primarily been gained from cell culture studies…

Previous studies, mostly in mice and men, have shown that senescent cells accumulate with age, with few studies in other species (eg, baboons)…

The theory that accumulated senescent cells create a microenvironment that is favorable for tumor development and growth **was supported** by co-culture as well as xenograft studies. Senescent fibroblasts stimulate the growth of preneoplastic …

The identification of biomarkers for senescent cells is rather challenging as no known single marker is considered specific….

So far, the presence of cellular senescence **has not been confirmed** in the dog, although aging dogs develop several age-related diseases, in particular in the testis, that appear at least in some aspects similar to those in humans.
Consequentially, we hypothesized that senescent cells accumulate with age in canine testes and in the microenvironment of testicular tumors. Hereby, senescent cells could play a role in tumor proliferation via paracrine activities. **We further expected** morphological changes that reflect the aging of canine testes (ie, decreased mean tubular diameter, increased relative collagen content, or I ncreased Leydig cell number per area). Thus, in the present study, **we tested** the 2 most established markers of senescence on canine tissues and correlated their expression with age-dependent morphological changes. Furthermore, we **asked whether** the proportion of senescent marker-positive cells changes in the microenvironment of testicular

tumors. The same **was investigated** for cryptorchid testes without tumors, as they are predisposed for tumor development, possibly due to premature aging.

Discussion:

Based on previous in vitro and ex vivo data, we hypothesized that senescent cells accumulate with age in canine testes and in the microenvironment of testicular tumors. Thus, senescent cells could play a role in tumor proliferation …

Reflecting the aging process, **we expected**…

Therefore, **we tested whether** the established markers of senescence, g H2AX and p21, could reveal an age-dependent increase of senescent cells in canine testes …

Supporting our data, Wang et al detected a sig- nificant yet small increase in…

We did not detect an increase in … First,… second… Third….

To our surprise, only few age-related morphological changes were found in old canine testes in our study…

Although this contradicts findings by Peters et al that spermatogenesis does not decrease significantly with age in dogs, …

Conclusions:

Our data did not support several previous hypotheses and expectations on structural changes as well as the role of senescence in aged canine dogs. Species-specific differences may be involved: for example, in contrast to humans, aged dogs presented a significantly increased mean number of Leydig cells per intertubular triangle (2.95-fold) and no decrease in the mean tubular epithelial area or tubular diameter compared to young dogs. Only a slight but significant decrease was found in the spermatogenesis score. **Different to what was expected,** no significant increase in interstitial collagen content was identified in testes of aged dogs. Although both p21 and gH2AX are considered senescence markers, only the relative number of p21-positive cells increased with age in healthy canine testes. **Again, opposed to our expectations based on previous in vitro studies**, we did not identify any evidence of a correlation between the age-associated accumulation of senescent cells and testicular tumor development.