EXPRESSION OF LH RECEPTOR IN CANINE CUTANEOUS MAST CELL TUMOR

GONADECTOMY INCREASES LH RECEPTOR EXPRESSION

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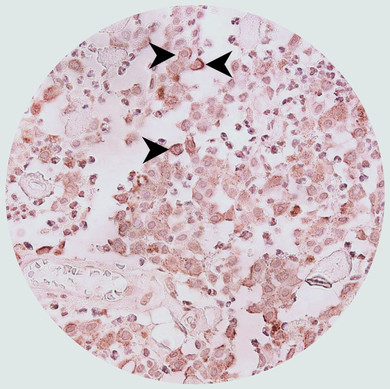


Figure 1. LHR- staining pattern I is characterized by membrane-associated staining (black arrowheads), with little cytoplasmic staining of neoplastic mast cells (black arrowheads).

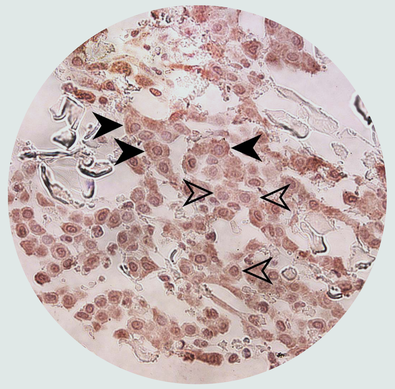


Figure 2. LHR- staining pattern II is characterized by low (open arrowheads) to medium (black arrowheads) cytoplasmic intensity staining with small granules in neoplastic mast cells.

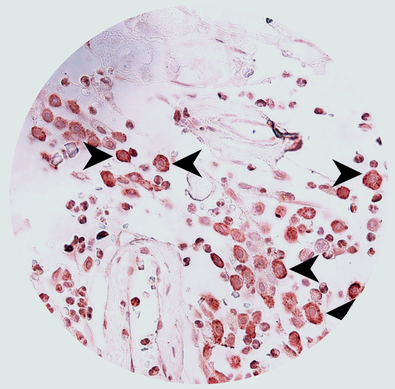
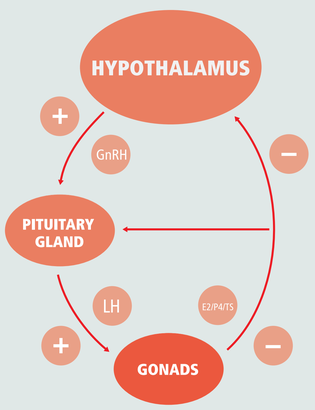


Figure 3. LHR- staining pattern III is characterized by high staining intensity (black arrowheads). Some neoplastic mast cells have staining of coalesced granules.

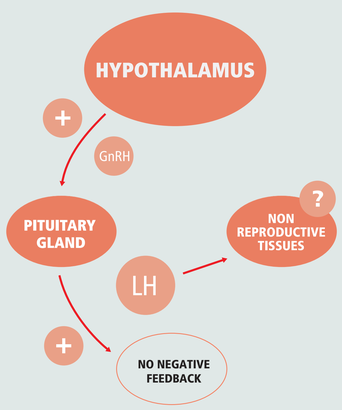
Introduction

Luteinizing hormone (LH) and his receptor are responsible for causing ovulation and stimulating the corpus luteum to produce progesterone in the female, whereas it causes testosterone production in the male. Furthermore, recent evidence demonstrates the existence of luteinizing hormone receptor (LHR) expression outside of the reproductive tract. Non-reproductive tissues that have been reported to express LHR include canine skin,[1] bladder and urethra,[2] thyroid gland,[3] and adrenal cortex.[4] Gonadectomy disrupts the negative feedback to the anterior pituitary, resulting in sustained, supraphysiologic circulating concentrations of LH that are more than twenty times the concentrations found in intact adult female dogs.[5] See **A — B .**

A • Normal Adult Mammal



B • Gonadectomized Mammal



GnRH= Gonadotropin releasing hormone, LH= Luteinizing hormone, E2=Estrogen, P4=Progesterone, TS=Testosterone

Mast cell tumor (MCT) is one of the most common neoplasms in dogs, accounting for 10–21% of all skin tumors in dogs.[6] It is assumed that breed, age, weight and gonadectomy may affect the MCT development.[7]

Objectives

Based upon the recent studies that have shown the expression of the LH receptor in several normal and neoplastic tissues, the mitogenic actions of LHR activation,[8] the association of LHR expression and increased metastasis in humans,[9] and the increased prevalence of MCT in gonadectomized female dogs,[10] we hypothesized:

- MCT could express LH receptors;
- MCT in gonadectomized dogs would express more LHR compared to MCT in intact dogs.

Materials and methods

Cutaneous mast cell tumors from intact dogs (n=6) and dogs gonadectomized under one year of age (n=5) with Patnaik grade 2 and Kiupel low grade were selected for immunohistochemistry.[11,12]
Images were digitally captured at 400X magnification using the Leica DMI6000 B microscope.
The percentage of positive LHR and c-KIT cells was determined from counting 200 cells within an area of the tumor with the highest density of cells.
Comparisons were made between intact and gonadectomized dogs for staining results of each antigen separately using a Student's t test. Significance was defined as $p<0.05$

Results

- Using immunohistochemistry, we identified LHR expression in all of the MCT evaluated. Results are summarized in **Table 1**.
- Three distinct patterns of LHR immunoexpression, similar to that of proto-oncogene c-Kit (mast cell growth factor receptor),[13] were identified. See **Figure 1 — Figure 2 — Figure 3**.
- Case demographic results from LHR and c-kit immunohistochemistry are summarized in **Table 2**.

Table 1. The percentage (mean±SD) of positive luteinizing hormone receptor (LHR) and c-Kit mast cells was determined from counting 200 cells within an area of the tumor with the highest density of cells. The cellular pattern of LHR and c-KIT expression was recorded for each positive cell.

Receptor	Neuter Status	Positive %	Pattern I %[a]	Pattern II %[a]	Pattern III %[a]
LHR					
	Intact (n=6)	**64.3 ± 4.2**	1.9 ± 1.4	**49.2 ± 8.4**	13.2 ± 9.7
	Gonadectomized (n=5)	**84.2 ± 8.7**	1.9 ± 0.8	**66.6 ± 15.3**	15.7 ± 12.1
		p=0.000	p=0.491	**p=0.020**	p=0.354

Receptor	Neuter Status	Positive %	Pattern I %[b]	Pattern II %[b]	Pattern III %[b]
c-Kit					
	Intact (n=6)	95.8 ± 2.7	13.3 ± 15.8	81.3 ± 15.4	1.7 ± 1.9
	Gonadectomized (n=5)	95.7 ± 2.0	17.6 ± 11.2	77.3 ± 8.9	1.2 ± 0.6
		p=0.487	p=0.313	p=0.313	p=0.307

[a]LHR cellular patterns of antigen distribution were categorized as follows: I- membranous; II- cytoplasmic (low to medium intensity); III- cytoplasmic (high intensity)
[b]c-KIT cellular patterns of antigen distribution were categorized as previously described.[13]

Table 2. Case demographics and results from luteinizing hormone receptor (LHR) and c-Kit immunohistochemistry. Cases selected for immunohistochemistry were Patnaik grade 2 and Kiupel low grade.

Breed	Age[a]	Status	Age at Gonadectomy[b]	Recurrence (Time)[c]	LHR Positive Cells %	c-Kit Positive Cells %
Heeler	8	SF	<6	No	94.5	82.5
Crossbreed	2	SF	6 to 12	No	71.5	73.5
Cocker Spaniel	6	SF	6 to 12	Yes (2 to 5)	86.5	77.5
Miniature Pinscher	11	SF	6 to 12	No	80.5	69.5
Lhasa Apso	11	SF	6 to 12	No	88.0	54.0
Boxer	7	M	Intact	No	69.5	89.5
Australian Terrier	2	M	Intact	No	61.0	73.0
Labrador Retriever	3	M	Intact	Yes (>6)	64.5	97.0
Labrador Retriever	8	F	Intact	No	62.0	92.0
Crossbreed	10	F	Intact	Yes (<1)	59.5	84.0
German Pointer	9	F	Intact	No	69.0	84.0

[a]In years; [b]In months; [c]Months after initial surgical excision; SF=Spayed Female M=Intact Male F=Intact Female

Discussion and conclusions

Although several studies previously have demonstrated expression of LHR in neoplastic tissues including canine lymphoma,[14] hemangiosarcoma,[15] and human thyroid gland adenoma,[16] this is the first study to demonstrate the expression of LHR in canine cutaneous MCT.
What role LHR plays, if any, in normal and neoplastic non-reproductive tissues is still under investigation. Some overlap between immunolocalization patterns of LHR and c-Kit was observed but it is unknown if the two receptors are functioning synergistically in MCT development or maintenance. With more research into the long-term health adverse effects associated with gonadectomy in dogs, evidence is becoming available to support other methods for sterilization.[17]

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