Genetic Mutations of Sox-2 and KDR in Canine Mast Cell Tumors

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Abstract

The present study aims to develop a procedure to experimentally investigate the presence and sequence of mutations within proto-oncogene KDR and transcription factor Sox-2 in Canine Mast Cell Tumors (MCTs). DNA was extracted from 20 samples of canine MCTs, and Quantitative PCR (qPCR) was performed. RNA was also extracted to investigate Sox-2 expression. The PCR product was utilized in gel electrophoresis and sent off for genetic sequencing. Analysis of melting curves, and DNA sequencing will characterize both KDR and Sox-2's role in varying grades of canine MCTs. MCTs are the most common form of skin neoplasia in canines, though, MCTs are present in other areas of the body as well. This data will aim to support the development of potential prognostic biomarkers and therapeutic drug agents.

Introduction

Canine Mast Cell Tumors (MCTs) are the most common form of skin cancer in canines. These tumors are graded on a scale of I-III based on their metastasis rate and severity. Some breeds of dogs are more susceptible to developing MCTs than others, most notably, Boxers and Labrador Retrievers.

Mutations in the c-KIT gene have been implicated in canine MCTs, c-KIT encodes for a receptor tyrosine kinase, or RTK, known as KIT. C-KIT receptors usually require their corresponding ligand to bind in order to activate. Activation occurs through phosphorylating and dimerizing. This causes proteins to begin attaching to the phosphorylated tyrosine groups which activate intracellular downstream pathways. When c-KIT becomes mutated, the receptor no longer required the ligand to bind in order to activate leading to unregulated cell growth.

When KIT was implicated in tumor development, Toceranib, an RTK inhibitor was tried as a treatment, and was successful against canines with c-KIT mutations and with those who did not. Since there are different types of RTKs found in the body that are relevant to cell growth, other RTK receptors could also be responsible, one being KDR or vascular endothelial growth factor receptor 2 (VEGFR2.) Increased VEGFR2 expression and mutations in the KDR gene have both been implicated in human cancers as well as MCTs.

More recent studies have discovered that some canine MCTs contain cancerous stem-like cells if mutations in embryonic transcription factors such as sex-determining region Y box 2 (Sox-2) allow it. It was found that a high Sox-2 immunopositivity correlated with higher grade MCTs, regardless of dog breed. Previous research was able to implicate Sox-2 in the development of MCTs, as well as discovering Sox-2 as a potential prognostic biomarker as Sox-2 protein expression correlated with higher graded MCTs but not dog breed. Sox-2 is a stem cell gene, any expression is indicative of anaplasia. The present study aims to identify if KDR and Sox-2 mutations and correlate expression of Sox-2 to tumor grade and malignancy.

Methods

44 Formalin-Fixed-Paraffin-Embedded (FFPE) were donated by the University of Pennsylvania's School of Veterinary Medicine Department of Clinical Pathology. These samples included various dog breeds as well as varying MCT grades ranging from I to III. Non-MCT lesions such as melanocytes and dermal scars were also included which served as controls. 20 samples were utilized and labeled A-T.

DNA was extracted with the Qiagen Nucleopsin DNA FFPE XS kit per the manufacturer's protocols and stored frozen until ready for qPCR. qPCR was run via a LightCycler® with primers for GAPDH serving as a control. Biotium's FastEvaGreen qPCR MasterMix was also utilized per manufacturer procedure. KDR primers and Sox-2 primers (purchased from IDT Technologies) were added to the PCR mix to amplify the correct sequences of DNA. Amplification and Melting curves were recorded for each qPCR.

PCR product was run on a 2% Agarose Gel containing GelRed dye immersed in 1x TBE. Carolina Biological PBR322/BstNI ladder was utilized as a molecular weight marker. New England BioLabs' Gel Loading Purple (6x) was utilized for PCR Product loading into wells. Gels were run for approximately 20-30 minutes and were visualized under a UV Transilluminator and each band was cut out and stored. Product was then extracted using QIAquick Gel Extraction and PCR Cleanup Kit and sent out to Genewiz (Sanger) for sequencing.

Results

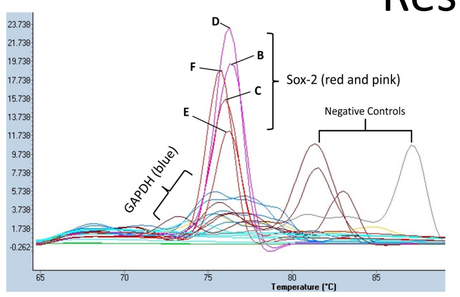


Figure 1: Sox-2 + GAPDH Melt Curve

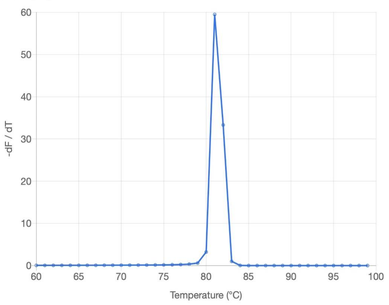


Figure 2: Predicted Sox-2 Melt Curve

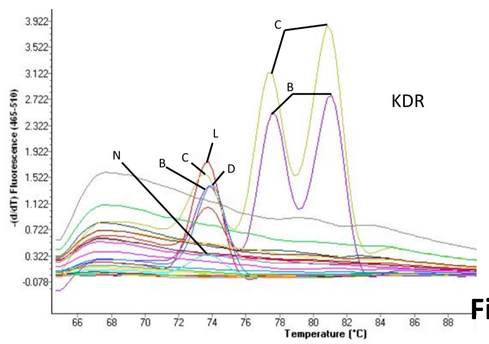
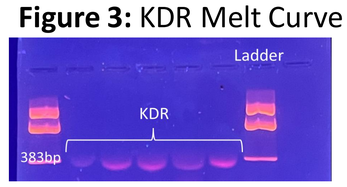


Figure 3: KDR Melt Curve



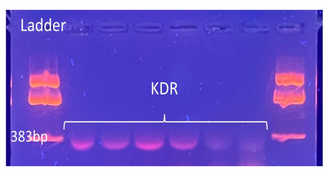


Figure 5: KDR Gel

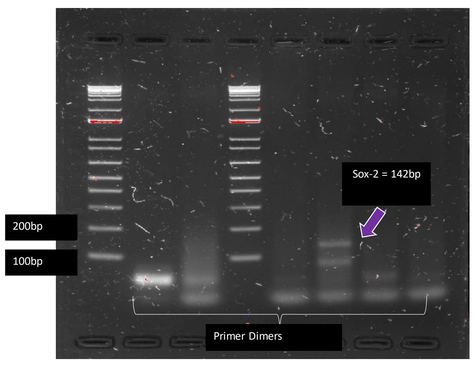


Figure 6: Sox-2 RNA Gel

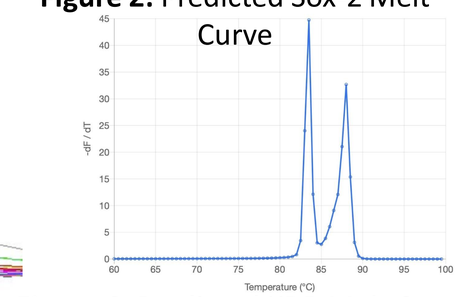


Figure 4: Predicted KDR Melt Curve

Discussion

Theoretically, if Sox-2 and KDR were not mutated in the canine MCT samples, all melting curve peaks would match very closely to that of the predicted melt curves. (Fig. 2+4) Typically, a melting point curve is a way to confirm the correct sequence had been amplified. When comparing the Sox-2 melt curve (Fig. 1) to the Predicted (Fig. 2) both are a tall skinny curve at around the same melting temperature. The same is true for KDR, both the experimental (Fig. 3) and predicted (Fig. 4) show two curves at similar melting temperatures. This indicates that the correct sequence was amplified for both KDR and Sox-2. GAPDH was used as a control while running Sox-2. In Sox-2's melt curve the melting point temperature varies between samples, typically this occurs due to differences in nucleic acid sequences. Sequence changes occur due to mutations in the DNA.

KDR PCR product was visualized via Gel Electrophoresis (Fig 5). At approximately 390bp one singular band was seen for all samples. If any insertion or deletion mutations were present within a sample, the band would be elsewhere due to a change in number of base pairs. However, this does not mean other types of mutation are not present (insertion, deletions.) DNA sequencing would be needed to identify these mutants, unfortunately sequencing was unsuccessful. In the RNA Gel Electrophoresis, 3 of 10 samples expressed Sox-2 visualized by banding at 142bp, although 2 of 3 had very faint banding. Expression of Sox-2 is indicative of de-differentiated cells able to multiply indefinitely, also known as anaplasia.

Conclusion

The present study successfully extracted DNA from FFPE canine MCTs and amplified both Sox-2 and KDR through qPCR. Though sequencing was unsuccessful, mutations in genome for Sox-2 are likely, based on the melting point analysis. Suggesting Sox-2 mutation play a role in canine MCT development.

After improvement and tweaking of current procedure, DNA and RNA sequencing will be reattempted to identify mutations focusing on Sox-2. Immunohistochemistry and Western Blots will be attempted in the future. The continuation of this study will potentially aid the future development of prognostic biomarkers and drug therapies.

Acknowledgement

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