RAS Gene Hot-Spot Mutations in Canine Neoplasias


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Abstract

Point mutations in the cellular homologues \textit{HRAS}, \textit{KRAS2}, and \textit{NRAS} of the viral Harvey and Kirsten rat sarcoma virus oncogenes are commonly involved in the onset of malignancies in humans and other species such as dog, mouse, and rat. Most often, three particular hot-spot codons are affected, with one amino acid exchange being sufficient for the induction of tumor growth. While \textit{RAS} genes have been shown to play an important role in canine tumors such as non-small lung cell carcinomas, data about \textit{RAS} mutations in canine fibrosarcomas as well as \textit{KRAS2} mutations in canine melanomas is sparse. To increase the number of tumors examined, we recently screened 13 canine fibrosarcomas and 11 canine melanomas for point mutations, particularly within the mutational hot spots. The results were compared to the already existing data from other studies about these tumors in dogs.

A family of genes often involved in human tumors are the well-characterized \textit{RAS} genes, which comprise \textit{HRAS}, \textit{KRAS2}, and \textit{NRAS}, coding for closely related, small, 189 amino acid, 21 kDa, membrane-bound, intracellular proteins. The human cellular \textit{HRAS} and \textit{KRAS2} genes were identified to be homologues of the Harvey and Kirsten rat sarcoma virus oncogenes \textit{v-Ha-ras} and \textit{v-Ki-ras2}, respectively (Der et al. 1982; Parada et al. 1982), with \textit{NRAS} being only weakly homologous to both \textit{v-Ha-ras} and \textit{v-Ki-ras2} (a \textit{v-N-ras} gene has not been described) (Shimizu et al. 1983). \textit{Ras} genes have been found in a variety of mammals, showing high sequence similarity across species, with sequence variation most often not affecting the amino acid sequence of the encoded proteins (Watzinger et al. 1998).

The \textit{RAS} proteins function in relaying mitogenic growth signals into the cytoplasm and nucleus, influencing proliferation, differentiation, transformation, and apoptosis of cells (Watzinger and Lion 1999). Regulation of \textit{RAS} protein activity occurs through intrinsic GTPase activity in the wild-type \textit{RAS}, which switches the protein from an active (guanosine triphosphate [GTP]-bound) to an inactive (guanosine diphosphate [GDP]-bound) state. Point mutations in a number of particular hot-spot codons in exon 1 (mostly codons 12 and 13) and exon 2 (mostly codon 61) lead to diminished GTPase activity, bringing about constant signal transduction and facilitating uncontrolled cell division and tumor growth (Park 1995).

Alterations in \textit{RAS} genes are among the most important incidents in the onset of malignancies in humans (Arber 1999; Hahn et al. 1994), and have been described in dog, mouse, and rat, among others. Studies indicate that in man, up to 13% of brain tumors, 30% of lung tumors, 30% of liver tumors, 30% of acute myelogenous leukemia, 53% of follicular and 60% of undifferentiated papillary thyroid tumors, 50% of tumors of the gastrointestinal tract, and 90% of pancreatic tumors are affected by a mutation in the hot-spot codons of one of the three known \textit{RAS} genes (Bos 1989; Knapp and Waters 1997; Spandidos et al. 2002; Tang et al. 2002).

Studies about the involvement of \textit{RAS} genes in canine tumors have been performed by a number of groups investigating several types of tumors. Gumerlock et al. (1989) described the formation of activated \textit{NRAS} through the substitution of glycine by aspartatic acid at position 12 of the protein in a case of a gamma radiation-induced canine acute nonlymphocytic leukemia.

\textit{KRAS2} activation was observed in non-small cell lung cancer of the dog (Kraegel et al. 1992). Out of 21 tumors, which included adenocarcinomas, adenosquamous carcinomas, and one large cell carcinoma, 5 were shown to be affected by mutations mostly of codon 12 of the \textit{KRAS2} gene, being similar to the overall frequency of \textit{KRAS2} involvement in non-small cell lung cancer in man (25%). This was confirmed by a later study investigating a wide range of...
canine lung tumors where 19 out of 117 tumors (16%) showed KRAS2 alterations in the hot-spot codons (Griffey et al. 1998). On the other hand, NRAS was shown to be infrequently activated in canine malignant lymphomas, with only 1 from 28 examined cases showing an amino acid substitution from glycine to aspartate at position 13 (Edwards et al. 1993).

Similar to malignant lymphomas, RAS gene mutations at the hot-spot loci were shown to be rarely or not involved in canine mammary tumors (Castagnaro 1995; Mayr et al. 1998). Furthermore, Watzinger et al. (1998) have shown in a variety of canine tumors that RAS genes are, compared to humans, rather infrequently involved in the onset of malignancies. In that study, only three fibrosarcomas were included, none of which showed RAS gene alterations. Since Guerrero et al. (2002) showed that fibrosarcomas can be induced in nude mice by subcutaneously injecting transfected fibroblasts with KRAS2 point mutations in codon 12, we recently screened a larger number of 13 canine fibrosarcomas for KRAS2 and NRAS mutations in the particular hot-spot codons. In addition, we also recently screened 11 canine melanomas for KRAS2 and NRAS mutations (Murua Escobar et al. 2004). However, none of the screened tumors showed the characteristic RAS alterations in the hot-spot codons. A low rate of NRAS involvement in canine melanomas has been shown before, with 2 of 16 tumors showing NRAS mutations (Mayr et al. 2003).

In summary, the data from the available studies on canine fibrosarcomas and melanomas (Mayr et al. 2003; Murua Escobar et al. 2004; Watzinger et al. 2001) strongly indicate that KRAS2 and NRAS mutations at the hot-spot loci are essentially very rare in the investigated canine tumor entities. To the best of our knowledge, from the total number of 32 screened canine fibrosarcomas and 17 screened canine melanomas, only 2 melanoma samples have been found to have exon 61 of the NRAS gene affected. For KRAS2, no mutations in the hot-spot codons have been found. However, to allow for a comparison of these canine tumors with research results from, for example, man and mouse, with vast amounts of data being available, a larger number of canine tumors will have to be screened in the future, as it is still too early to draw conclusions from the relatively small number of canine tumors examined.

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References


