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Cortisol-secreting adrenocortical tumours in dogs and their relevance for human medicine

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ABSTRACT

Spontaneous cortisol-secreting adrenocortical tumours in pet dogs are an attractive animal model for their human counterparts. Adrenal morphology and function are similar in dogs and humans, and adrenocortical tumours have comparable clinical and pathological characteristics. Their relatively high incidence in pet dogs represents a potential source of adrenocortical tumour tissue to facilitate research. The molecular characteristics of canine cortisol-secreting adrenocortical tumours suggest that they will be useful for the study of angiogenesis, the cAMP/protein kinase A pathway, and the role of Steroidogenic Factor-1 in adrenal tumourigenesis. Pet dogs with spontaneous cortisol-secreting adrenocortical tumours may also be useful in clinical testing of new drugs and in investigating the molecular background of adrenocortical tumours.

1. Introduction

The potential for pet dogs as animal models in cancer research has been foreseen by many investigators over de past decades (Paoloni and Khanna, 2008; Knapp and Waters, 1997; Hahn et al., 1994). Advances in technology and the availability of the canine genome have recently made it possible to apply the high-throughput methodologies to investigate canine cancer. Comparisons of the canine and human genomes have demonstrated strong similarities and a significant homology between them for recognized cancer-associated genes (Gordon et al., 2009; Parker et al., 2010).

Spontaneous cortisol-secreting adrenocortical tumours (C-ACTs) in pet dogs share biological and clinical features with its human counterparts and are a subject to similar carcinogens (Galac et al., 2010a). Furthermore, the treatment of choice for canine C-ACT is adrenalectomy (Naan et al., 2013; van Sluijs et al., 1995), which can generate substantial amounts of adrenocortical tumour tissue for *in vitro* research purposes. Another advantage of cancer research in dogs is that dogs age approximately 5–7 times faster than humans and the disease progression is faster, which shortens the span to trial end points. Also, euthanasia is possible and accepted to prevent prolonged suffering (Cekanova and Rathore, 2014; Ranieri et al., 2013).

In order to be able to use pet dogs as an animal model of adrenocortical tumourigenesis, molecular profiling of canine C-ACTs is essential. In this review, the most relevant molecular pathways for human adrenocortical tumourigenesis will be presented.

2. Molecular characteristics of canine C-ACTs

Our early studies of C-ACTs (adenomas and carcinomas) focused on ACTH-independent cortisol secretion. We initially hypothesized that increased steroidogenic enzyme expression might provide an explanation for the autonomous hypercortisolism, but no difference could be demonstrated in the relative expression of mRNA encoding steroidogenic enzymes between tumour tissues and normal adrenals (Galac et al., 2010b). Aberrant receptors coupled to the Gs-protein, such as luteinizing hormone (LH) receptor, gastric inhibitory polypeptide (GIP) receptor, all 3 types of vasopressin receptor (V_{1a}, V_{1b}, V_2), and dopamine and somatostatin receptor, were shown to play a minor role (Galac et al., 2010c; Kool et al., 2015a). The relative expression of the melanocortin receptor 2 (MC2R) was significantly lower in carcinomas than in adenomas and normal adrenals, and our tentative explanation was that this is a result of dedifferentiation of carcinoma cells (Galac et al., 2010b).

Abbreviations: C-ACT, cortisol-secreting adrenocortical tumour; IGF, insulin-like-growth factor; MC2R, melanocortin receptor; SF-1, Steroidogenic factor-1; VEGF, vascular endothelial growth factor; GNAS, G protein alpha subunit gene; PI3K, phosphatidylinositol-3-kinase.

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The pathognomonic changes in the insulin-like-growth factor (IGF) system in human adrenal cancer (Giordano et al., 2003; Ribeiro and Latronico, 2012), was a trigger to study IGF signalling in canine C-ACTs. The relative mRNA expressions of IGF-2 and IGF receptor 1 (IGFR1) between normal adrenal tissue and tumour tissue did not differ in dogs (Kool et al., 2015b). Among the genes encoding for members of the IGF family, the higher relative expression of IGF binding protein 2 (IGFBP2) in tumour tissue compared to normal adrenocortical tissue was the only remarkable finding. Enhanced protein levels of IGFBP2 in humans are associated with malignancy, but no differences in mRNA expression were detected (Boulle et al., 1998). In dogs, relative expression of mRNA encoding IGFBP2 did not differ between adenomas and carcinomas. Recently it was proposed that comparative studies in humans and dogs could provide a novel strategy to distinguish driver and passenger alterations in carcinogenesis (Ji et al., 2010). Although this was based on genomic amplifications and deletions, the same principle may apply to other types of studies. Studying dogs with C-ACTs may strengthen the notion that the IGF system is merely a passenger alteration.

The lack of changes in the IGF system in dogs does not preclude activation of the downstream phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway, which became another subject of our study. The results indicated PI3K activation in carcinomas, but not in adenomas (Kool et al., 2015b). No amino acid changing mutations were detected in PTEN or PI3K catalytic subunit (PI3KCA), but there was a tendency toward a higher expression of epidermal growth factor (EGF) receptor family member erythroblastic leukaemia viral oncogene homologue 2 (ERBB2) in carcinomas. Also, two of the pathway's target genes, inhibitor of differentiation 1 and 2 (ID1, ID2), were expressed at higher levels in the group of carcinomas with short survival after adrenalectomy. The ID proteins are thought to keep cells in a poorly differentiated, proliferative state (Nair et al., 2014). These results suggest that PI3K and/or ID signalling may contribute to the uncontrolled growth of canine C-ACTs. The relevance of this finding to human adrenal tumourigenesis is so far unknown, as no studies have yet evaluated ERBB2, ID1, and ID2 expression in human adrenocortical tumours.

With regard to the use of the pet dog as an animal model, the angiogenesis, Steroidogenic Factor-1 (SF-1) expression and the mutations in the MC2R-cyclic AMP (cAMP)-protein kinase A (PKA) pathway seem to be the most interesting molecular pathways and will be discussed in detail.

2.1. Angiogenesis in canine C-ACTs

Angiogenesis is recognized as an important factor in tumour development and metastasis. By means of intra-temporal angiogenic feedback loops, tumours may activate angiogenesis and provide themselves with the nutrients and oxygen necessary to grow beyond a certain size.

In canine C-ACTs markedly increased relative expression of angiopoetin 2 (Ang2) has been detected, similar to findings in humans (Kool et al., 2014; Giordano et al., 2003). In addition to the full-length Ang2, its splice variant Ang2-443 has also been demonstrated in canine adrenocortical tissue (Fig. 1A) and the magnitude of change in relative mRNA expression of Ang2-443 even exceeded that of full-length Ang2 when comparison was made between C-ACTs and normal adrenocortical tissue and between adenomas and carcinomas. At the same time, the Ang2-to-Ang1 ratio in C-ACTs increased (Fig. 1B) (Kool et al., 2013) and was highest in carcinomas, which has been interpreted as a shift of the angiogenic balance towards a pro-angiogenic state (Tait and Jones, 2004). Immunohistochemical staining showed positive Ang2 expression in the adenoma and carcinoma tumour cells. Notably, significant Ang2 staining in the vascular endothelial lining was limited to carcinomas, possibly indicating a role in carcinogenesis (Fig. 1C). Additional evidence for the functional role of Ang2 is the presence of vascular endothelial growth factor (VEGF). As in humans, abundant expression of VEGF was documented in canine C-ACTs (Turner et al., 2003). Although the relative VEGF mRNA expression was not different between C-ACTs and normal adrenals, its presence is essential for the angiogenesis-stimulating effect of Ang2. These results suggest that the Ang family may be an interesting target for medical intervention. No studies in human adrenal tumours have yet reported on the use of angiogenesis inhibitors, but the use of anti-angiogenic drugs is one of the most rapidly emerging strategies in cancer medicine (Vasudev and Reynolds, 2014; El-Kenawi and El-Remessy, 2013). In dogs, targeting of the Ang2-Tie2 pathway by a selective antagonist such as Ang2 traps or monoclonal antibodies (Huang et al., 2010; Eroglu et al., 2013) may hold promise as an adjunctive therapy and warrants further investigation. Pet dogs with metastasised C-ACT can serve as a suitable animal model.

2.2. The role of SF-1 in canine adrenocortical tumourigenesis

SF-1 is a known cAMP downstream effector with a profound influence on steroidogenesis and adrenocortical growth. Its relevance in human adrenocortical biology and development of adrenocortical neoplasia has been clearly demonstrated (Lalli, 2010; Lalli et al., 2013). In the dog, the relative SF-1 mRNA expression in normal adrenals and C-ACTs does not differ, however, in a subset of carcinomas with recurrence of ACTH-independent hypercortisolism within 2.5 years after adrenalectomy the relative mRNA expression of SF-1 was significantly higher than in dogs in remission at least 2.5 years after surgery. In the majority of dogs with a poor outcome, liver and/or lung metastases were detected. Theoretically, this effect could be mediated by an SF-1-induced increased expression of the angiogenic gene Ang2 (Ferraz-de-Souza et al., 2011). Further investigation of SF-1-dependent activation of Ang2 transcription is warranted. Immunohistochemistry with a polyclonal antibody against SF-1 revealed predominantly nuclear staining in cortical zones of normal adrenals (Fig. 2A) as well as in C-ACTs (Fig. 2C) (Galac et al., 2014). Also a weak cytoplasmic signal was visible and this has been ascribed to the aspecificity of the polyclonal antibody. Similar has been noted in human ACC tissue and the monoclonal antibody against SF-1 has been proven superior to polyclonal (Duregon et al., 2013). Unfortunately, the monoclonal antibody against SF-1 is not immunocompetent in canine spp. An international study to test the utility of SF-1 mRNA and/or protein expression as a prognostic marker in canine C-ACTs, comparable to the study of Sbiera et al. (Sbiera et al., 2010), is ongoing in dogs with C-ACTs.

In conclusion, there is a link between mRNA abundances encoding SF-1 and poor clinical outcome. At first glance this may seem contradictory, but it could be related to the functional role of SF-1, which clearly depends on the cellular context (Parker et al., 2002; Gummow et al., 2006). In the normal adult differentiated adrenocortical cell, the major role of SF-1 is the regulation of steroidogenesis. In foetal adrenal development, SF-1 stimulates proliferation of non-differentiated cells, resulting in adrenal growth independent of steroid synthesis (Gummow et al., 2006). In H295R cells, an increased SF-1 dosage modulates steroid secretion profile and reinforces cellular differentiation towards a foetal adrenal phenotype (Doghman et al., 2007). If similar is the case in canine carcinomas, remains to be a elucidated.

The high SF-1 mRNA expression in carcinomas with early recurrence might indicate that SF-1 could be a useful target for

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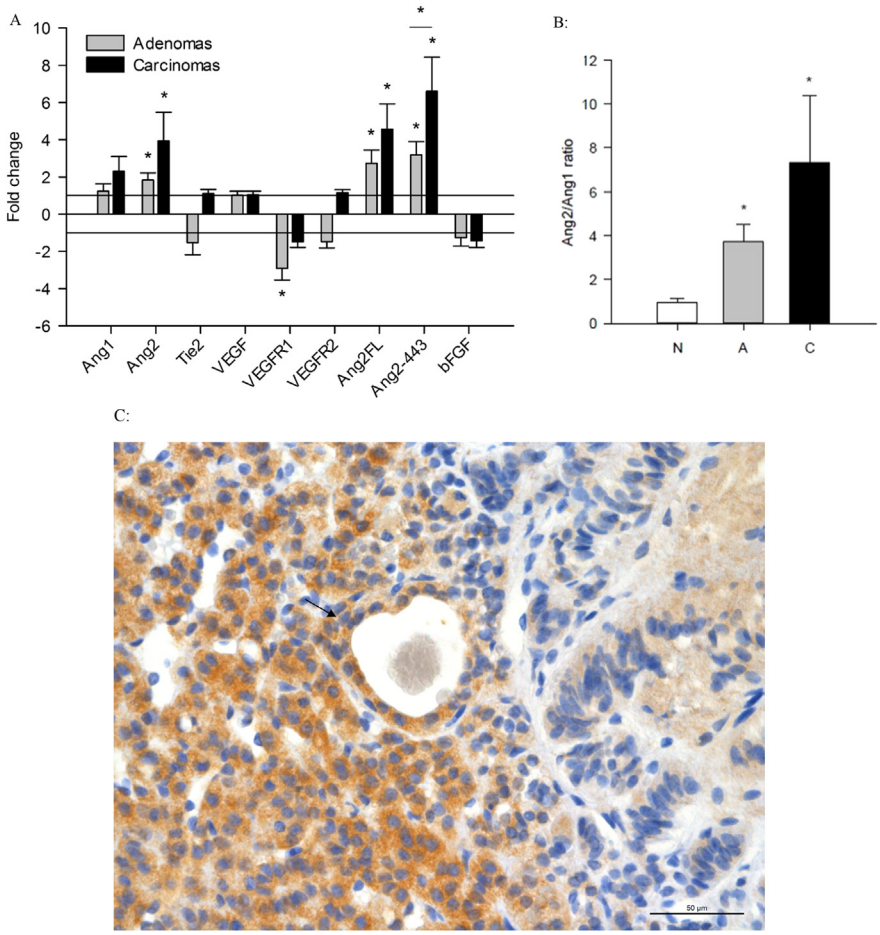

Fig. 1. 1A: Relative mRNA expression of target genes in adenomas and carcinomas compared with that in normal adrenal glands, as measured by qPCR and calculated using the $2^{\Delta\Delta}$Ct method. Bars represent mean ± SEM. Significant changes ($P < 0.05$) are indicated by *. Legend: Ang1: Angiopoietin 1, Ang2: Angiopoietin 2, Ang2FL: Angiopoietin 2-Full-length variant, Ang2-443: Angiopoietin 2-443, Tie-2: Angiopoietin receptor Tie2, bFGF: b-Fibroblast Growth Factor, VEGF: Vascular Endothelial Growth Factor, VEGFR1: VEGF receptor 1, VEGFR2: VEGF-receptor 2. 1B: Ang2—Ang1 ratio in normal adrenals (N), adenomas (A), and carcinomas (C). The significantly higher Ang2/Ang1 ratios in A and C are indicated by *. 1C: Representative example of immunohistochemical expression of Angiopoietin 2 (Ang2) in cortisol-secreting carcinoma. A polyclonal rabbit-anti-human anti-Ang2FL antibody was used after being validated for dogs (Ab6583, AbCam). In normal adrenal cortical zona glomerulosa weak immunostaining was detected. Carcinoma tissue represent with cytoplasmic granular staining including a signal in the vascular endothelial lining (arrow). Modified from Kool et al., 2014. Expression of angiogenesis-related genes in canine cortisol-secreting adrenocortical tumours. Domest. Anim. Endocrinol. 47, 73—82.

treatment of pet dogs with SF-1 inverse agonists (Piu and Del Tredici, 2010). These isoquinolone compounds have been shown to selectively inhibit cell proliferation in a human adrenocortical tumour cell line, and to suppress steroid hormone secretion (Doghman and Lalli, 2009; Doghman et al., 2010). Drugs targeting SF-1 would be beneficial in medical management of at least a subgroup of canine C-ACTs, i.e., with inoperable masses due to local invasion and/or metastasis. *In vitro* studies on primary canine C-ACT cell cultures are needed to evaluate their effect on steroidogenesis and proliferation and to reveal their therapeutic potential. We are currently carrying out *in vitro* testing with various SF-1 inverse agonists in canine primary adrenocortical tumour cell suspensions, comparable to the studies performed in the human cell line H295R (Doghman et al., 2009). Hopefully, the results may also be extrapolated to human medicine.

The link between cAMP and SF-1 activity may be further elucidated in a canine *in vitro* model. While cAMP-induced regulation of SF-1 expression has been postulated (Schimmer and White, 2010), other studies have contradicted this hypothesis (Nomura et al., 1998; Chau et al., 1997). Alternatively, cAMP signalling may recruit SF-1 on a posttranscriptional level. A mechanism in which SF-1 is activated by the binding of phosphatidic acid upon cAMP signalling has been suggested (Urs et al., 2006; Li et al., 2007). Therefore, phospholipid profiling in primary cultures of C-ACTs could prove valuable in finding the explanation.

2.3. Mutations in the MC2R-cAMP-PKA pathway in canine C-ACTs

One of the main characteristics of C-ACTs is ACTH-independent cortisol secretion. A pathway with the potential to explain this so-called autonomous action is the cAMP-PKA signalling pathway, including its initiator, MC2R (Gallo-Payet and Payet, 2003).

Mutation analysis of MC2R in canine C-ACTs revealed the presence of a V291I missense mutation in 3 dogs (Kool et al., 2013). This mutation has not been previously reported and this substitution is not likely to have a functional effect on the receptor, as valine and isoleucine are alike in polarity and charge. Moreover, in the human MC2R isoleucine and not valine is the consensus amino acid. Otherwise no mutations in the MC2R were found, corresponding to the situation in humans, where activating MC2R mutations have never been identified (Latronico et al., 1995).

Mutation analysis of the cAMP pathway revealed stimulatory G protein alpha subunit gene (GNAS) missense mutations in about one-third of dogs (4 of 14 adenomas and 10 of 30 carcinomas) (Kool et al., 2013). Eleven of the 14 missense mutations were located in codon 201. They were present in 8 carcinomas and 3 adenomas and

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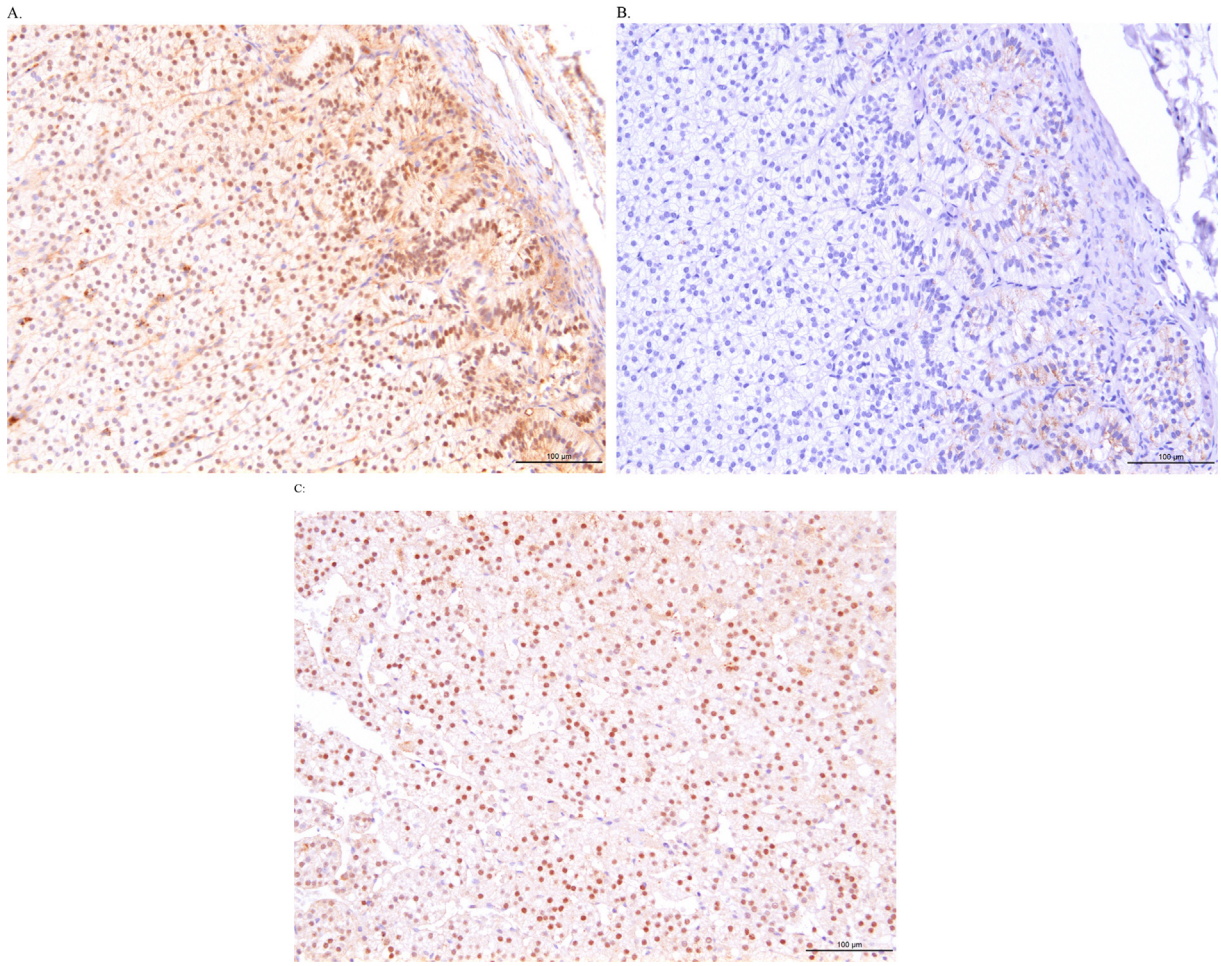

Fig. 2. Representative examples of immunohistochemical expression of Steroidogenic Factor-1 (SF-1) in normal canine adrenal gland (A, B is staining with SF-1 blocking peptide LS-P5534, LifeSpan Biosciences, Inc.) and cortisol-secreting adenoma (C). A polyclonal rabbit-anti-human anti−SF−1 antibody was used after being validated for dogs (LS-A5534, LifeSpan Biosciences, Inc.). Positive nuclear signal is present in normal adrenal cortical zones (A) and cortisol-secreting adrenocortical tumour (C) and there is some weak cytoplasmic staining, interpreted as an unspecific signal. Staining with blocking peptide (LS-E29078, LifeSpan Biosciences, Inc.), resulted in the disappearance of the nuclear signal, while weak cytoplasmic staining is still visible (B).

comprised the following substitutions: R201C, R201H, R201S, and R201L. A missense mutation in codon 203 (L203P) was present in 1 adenoma and missense mutations in codon 227 were present in 2 carcinomas (Q227H and Q227R) (Table 1). All GNAS mutations detected in the canine C-ACTs were reported previously in humans and were found to cause constitutive activation of cAMP signalling, as they lead to activation of $G_s$alpha (Kobayashi et al., 2000; Nishihara et al., 2009; Hayward et al., 2001). cAMP is the main cellular signal for inducing cortisol secretion (Gallo-Payet and Payet, 2003). Therefore, the activating GNAS mutations in the canine ATs, resulting in constitutive cAMP production, may explain the ACTH-independent cortisol production. Apart from cortisol production, increased cAMP signalling is also known to play a role in adrenal tumourigenesis (Almeida and Stratakis, 2011). Activating mutations in *GNAS* induce tumour formation in cAMP-sensitive tissue types by increasing cell proliferation. The mutated *GNAS* is referred to as the gsp oncogene (Lyons et al., 1990). In humans, activating *GNAS* mutations are associated with McCune Albright syndrome, where they result in ACTH-independent macronodular adrenocortical hyperplasia (AIMAH) and hypercortisolism (Lumbroso et al., 2004; Libe et al., 2005), and recent research demonstrated the presence of this mutation in ACTH-independent adrenocortical adenomas (Sato et al., 2014). The two activating mutations described in humans (R201C and R201H) are also the

Table 1
Overview of all missense mutations of *GNAS* in 44 canine cortisol-secreting adrenocortical tumours (Kool et al., 2013). All nucleotide positions are based on the mRNA sequence (NM_001003263.1).

Mutation	Nucleotide	Codon	Base pair change	Amino acid change	Number of ATs
R201C	954	201	**C**GT > **T**GT	Arg > Cys	5
R201H	955	201	C**G**T > C**A**T	Arg > His	4
R201S	954	201	**C**GT > **A**GT	Arg > Ser	1
R201L	955	201	C**G**T > C**T**T	Arg > Leu	1
Q227H	1034	227	CA**G** > CA**T**	Gln > His	1
Q227R	1033	227	CA**G** > C**G**G	Gln > Arg	1
L203P	961	203	**C**T**G** > **C**C**G**	Leu > Pro	1

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most common ones in dogs (Table 1). In addition to the GNAS mutations, a mutation of the gene encoding the catalytic subunit (C subunit) of PKA (PRKACA) in more than 50% of investigated tumour samples has been reported in human adenomas (Beuschlein et al., 2014; Cao et al., 2014; Sato et al., 2014). In canine C-ACTs, no mutations affecting the amino-acid sequence were found in PRKACA (*own unpublished observation*).

In all investigated canine C-ACT samples, the GNAS splice variant was present and was analogous to human transcript variant 3 (Bray et al., 1987). This transcript variant corresponds to a shorter GNAS (GNASS), which was found to be co-expressed with the long variant (GNASL) in nearly all cell types, although the relative amounts vary depending on the tissue type. In the human adrenal cortex, GNASL was found to be the predominant isoform (Mumby et al., 1986). Both variants induce cAMP production, but some investigations have indicated differences in their activity, affinity of GDP binding, and receptor interaction (Walseth et al., 1989; Graziano et al., 1989). However, whether these differences result in significant biological effects is still unclear (Bastepe, 2007).

In contrast with the significant role of *PRKAR1A* mutations in human and murine adrenal pathologies (Rothenbuhler and Stratakis, 2010), missense *PRKAR1A* mutations in the canine C-ACT cohort were not detected (Kool et al., 2013). There has been a single case report of a syndrome similar to human Carney complex occurring in a dog, but *PRKAR1A* was not altered (Adissu et al., 2010).

Constitutive cAMP/PKA mutations might provide an alternative route to activate Wnt signalling. The canonical Wnt activation, as denoted by nucleocytoplasmic β-catenin staining, is reported to be important in the pathogenesis of adrenal cancer in humans (Kim et al., 2009; Berthon et al., 2012; Simon and Hammer, 2012). In the canine cohort, Wnt-pathway activation was detected in 13 of 36 C-CATs. In two of these, activation could be explained by an amino acid changing mutation in the gene encoding β-catenin (CTNNB1). In the other C-ACTs, neither mutations nor increased ligand expression were detected, and the GNAS mutation was not associated with higher Wnt ligand expression. Consequently, the cause of Wnt activation in the C-ACTs negative for CTNNB1 and AXIN2 mutations remains unclear.

The mutations found in the MC2R-cAMP-PKA pathway provided the first possible explanation for steroid synthesis and proliferation in the affected subset of canine C-ACTs. While this was a welcome achievement with regard to the pathogenesis of C-ACTs, the question remains whether identification of mutations in the cAMP-PKA pathway could alter patient management. PKA signalling is universal in the body and agents that target PKA signalling are likely to have considerable toxicity. Inhibitors of cAMP signalling and/or PKA and its catalytic subunits exist *in vivo* (Aimes et al., 2000; Niswender et al., 2002), and it is to be expected that 'smart' PKA inhibitors, which only target adrenal PKA signalling, could be developed. Despite the fact that a C-ACT does not arise from the same mutation in pet dogs and humans, the dog could still fulfil the role of animal model in testing cAMP-PKA inhibitors, as long as the differences are taken into the account.

3. Conclusions

A pet dog offers several advantages as a model for human adrenocortical tumourigenesis: adrenal morphology and function in dogs and humans are highly homologous, both humans and dogs spontaneously develop C-ACTs, the clinical and pathological characteristics of C-ACTs are comparable, and the higher incidence of C-ACTs in dogs helps to facilitate research. Recent studies have demonstrated similarities in the relative expression of MC2R and transcription factor SF-1 between canine and human C-ACTs, and in both there are activating mutations of the cAMP-PKA signalling pathway. In those aspects, pet dogs with C-ACTs can be of great value as a model to evaluate new therapeutic options and to help elucidate the pathogenesis of C-ACT. Further research is needed to explore all aspects of the use of the pet dog as an animal model in adrenocortical tumourigenesis and to integrate the pet dog with spontaneous C-ACT in the research process and even in clinical trials, where applicable.

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References

Adissu, H.A., Hayes, G., Wood, G.A., Caswell, J.L., 2010. Cardiac myxosarcoma with adrenal adenoma and pituitary hyperplasia resembling Carney complex in a dog. Vet. Pathol. 47, 354–357.

Aimes, R.T., Hemmer, W., Taylor, S.S., 2000. Serine-53 at the tip of the glycine-rich loop of cAMP-dependent protein kinase: role in catalysis, P-site specificity, and interaction with inhibitors. Biochemistry 39, 8325–8332.

Almeida, M.Q., Stratakis, C.A., 2011. How does cAMP/protein kinase A signaling lead to tumors in the adrenal cortex and other tissues? Mol. Cell. Endocrinol. 336, 162–168.

Bastepe, M., 2007. The GNAS locus: quintessential complex gene encoding Gsalpha, XLalphas, and other imprinted transcripts. Curr. Genomics 8, 398–414.

Berthon, A., Martinez, A., Bertherat, J., Val, P., 2012. Wnt/beta-catenin signalling in adrenal physiology and tumour development. Mol. Cell. Endocrinol. 351, 87–95.

Beuschlein, F., Fassnacht, M., Assie, G., Calebiro, D., Stratakis, C.A., Osswald, A., Ronchi, C.L., Wieland, T., Sbiera, S., Faucz, F.R., Schaak, K., Schmittfull, A., Schwarzmayr, T., Barreau, O., Vezzosi, D., Rizk-Rabin, M., Zabel, U., Szarek, E., Salpea, P., Forlino, A., Vetro, A., Zuffardi, O., Kisker, C., Diener, S., Meitinger, T., Lohse, M.J., Reincke, M., Bertherat, J., Strom, T.M., Allolio, B., 2014. Constitutive activation of PKA catalytic subunit in adrenal Cushing's syndrome. N. Engl. J. Med. 370, 1019–1028.

Boulle, N., Logie, A., Gicquel, C., Perin, L., Le Bouc, Y., 1998. Increased levels of insulin-like growth factor II (IGF-II) and IGF-binding protein-2 are associated with malignancy in sporadic adrenocortical tumors. J. Clin. Endocrinol. Metab. 83, 1713–1720.

Bray, P., Carter, A., Guo, V., Puckett, C., Kamholz, J., Spiegel, A., Nirenberg, M., 1987. Human cDNA clones for an alpha subunit of Gi signal-transduction protein. Proc. Natl. Acad. Sci. U. S. A. 84, 5115–5119.

Cao, Y., He, M., Gao, Z., Peng, Y., Li, Y., Li, L., Zhou, W., Li, X., Zhong, X., Lei, Y., Su, T., Wang, H., Jiang, Y., Yang, L., Wei, W., Yang, X., Jiang, X., Liu, L., He, J., Ye, J., Wei, Q., Li, Y., Wang, W., Wang, J., Ning, G., 2014. Activating hotspot L205R mutation in PRKACA and adrenal Cushing's syndrome. Science 344, 913–917.

Cekanova, M., Rathore, K., 2014. Animal models and therapeutic molecular targets of cancer: utility and limitations. Drug Des. Devel. Ther. 8, 1911–1921.

Chau, Y.M., Crawford, P.A., Woodson, K.G., Polish, J.A., Olson, L.M., Sadovsky, Y., 1997. Role of steroidogenic-factor 1 in basal and 3′,5'-cyclic adenosine monophosphate-mediated regulation of cytochrome P450 side-chain cleavage enzyme in the mouse. Biol. Reprod. 57, 765–771.

Doghman, M., Karpova, T., Rodrigues, G.A., Arhatte, M., De Moura, J., Cavalli, L.R., Virolle, V., Barbry, P., Zambetti, G.P., Figueiredo, B.C., Heckert, L.L., Lalli, E., 2007. Increased steroidogenic factor-1 dosage triggers adrenocortical cell proliferation and cancer. Mol. Endocrinol. 21, 2968–2987.

Doghman, M., Lalli, E., 2009. A matter of dosage: SF-1 in adrenocortical development and cancer. Ann. Endocrinol. Paris 70, 148–152.

Doghman, M., Cazareth, J., Douguet, D., Madoux, F., Hodder, P., Lalli, E., 2009. Inhibition of adrenocortical carcinoma cell proliferation by steroidogenic factor-1 inverse agonists. J. Clin. Endocrinol. Metab. 94, 2178–2183.

Doghman, M., Madoux, F., Hodder, P., Lalli, E., 2010. Identification and characterization of steroidogenic factor-1 inverse agonists. Methods Enzymol. 485, 3–23.

Duregon, E., Volante, M., Giorcelli, J., Terzolo, M., Lalli, E., Papotti, M., 2013. Diagnostic and prognostic role of steroidogenic factor 1 in adrenocortical carcinoma: a validation study focusing on clinical and pathologic correlates. Hum. Pathol. 44, 822–828.

El-Kenawi, A.E., El-Remessy, A.B., 2013. Angiogenesis inhibitors in cancer therapy: mechanistic perspective on classification and treatment rationales. Br. J. Pharmacol. 170, 712–729.

Eroglu, Z., Stein, C.A., Pal, S.K., 2013. Targeting angiopoietin-2 signalling in cancer therapy. Expert Opin. Investig. Drugs 22, 813–825.

Ferraz-de-Souza, B., Lin, L., Shah, S., Jina, N., Hubank, M., Dattani, M.T., Achermann, J.C., 2011. ChIP-on-chip analysis reveals angiopoietin 2 (Ang2, ANGPT2) as a novel target of steroidogenic factor-1 (SF-1, NR5A1) in the human

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adrenal gland. FASEB J. 25, 1166–1175.
Galac, S., Reusch, C., Kooistra, H., Rijnberk, A., 2010a. Adrenals. In: Rijnberk, A., Kooistra, H. (Eds.), Clinical Endocrinology of Dogs and Cats. Schlutersche, Hannover, pp. 93–154.
Galac, S., Kool, M.M., Naan, E.C., Daminet, S., Mol, J.A., Kooistra, H.S., 2010b. Expression of the ACTH receptor, steroidogenic acute regulatory protein, and steroidogenic enzymes in canine cortisol-secreting adrenocortical tumors. Domest. Anim. Endocrinol. 39, 259–267.
Galac, S., Kars, V.J., Klarenbeek, S., Teerds, K.J., Mol, J.A., Kooistra, H.S., 2010c. Expression of receptors for luteinizing hormone, gastric-inhibitory polypeptide, and vasopressin in normal adrenal glands and cortisol-secreting adrenocortical tumors in dogs. Domest. Anim. Endocrinol. 39, 63–75.
Galac, S., Kool, M.M., van den Berg, M.F., Mol, J.A., Kooistra, H.S., 2014. Expression of steroidogenic factor 1 in canine cortisol-secreting adrenocortical tumors and normal adrenals. Domest. Anim. Endocrinol. 4, 1–5.
Gallo-Payet, N., Payet, M.D., 2003. Mechanism of action of ACTH: beyond cAMP. Microsc. Res. Tech. 61, 275–287.
Giordano, T.J., Thomas, D.G., Kuick, R., Lizyness, M., Misek, D.E., Smith, A.L., Sanders, D., Aljundi, R.T., Gauger, P.G., Thompson, N.W., Taylor, J.M., Hanash, S.M., 2003. Distinct transcriptional profiles of adrenocortical tumors uncovered by DNA microarray analysis. Am. J. Pathol. 162, 521–531.
Gordon, I., Paoloni, M., Mazcko, C., Khanna, C., 2009. The comparative oncology trials consortium: using spontaneously occurring cancers in dogs to inform the cancer drug development pathway. PLoS Med. 6, e1000161.
Graziano, M.P., Freissmuth, M., Gilman, A.G., 1989. Expression of Gs alpha in *Escherichia coli*. Purification and properties of two forms of the protein. J. Biol. Chem. 264, 409–418.
Gummow, B.M., Scheys, J.O., Cancelli, V.R., Hammer, G.D., 2006. Reciprocal regulation of a glucocorticoid receptor-steroidogenic factor-1 transcription complex on the Dax-1 promoter by glucocorticoids and adrenocorticotropic hormone in the adrenal cortex. Mol. Endocrinol. 20, 2711–2723.
Hahn, K.A., Bravo, L., Adams, W.H., Frazier, D.L., 1994. Naturally occurring tumors in dogs as comparative models for cancer therapy research. In Vivo 8, 133–143.
Hayward, B.E., Barlier, A., Korbonits, M., Grossman, A.B., Jacquet, P., Enjalbert, A., Bonthron, D.T., 2001. Imprinting of the G(s)alpha gene GNAS1 in the pathogenesis of acromegaly. J. Clin. Invest. 107, R31–R36.
Huang, H., Bhat, A., Woodnutt, G., Lappe, R., 2010. Targeting the ANGPT-TIE2 pathway in malignancy. Nat. Rev. Cancer 10, 575–585.
Ji, X., Tang, J., Halberg, R., Busam, D., Ferriera, S., Peña, M.M., Venkataramu, C., Yeatman, T.J., Zhao, S., 2010. Distinguishing between cancer driver and passenger gene alteration candidates via cross-species comparison: a pilot study. BMC Cancer 10, 426.
Kim, A., Giordano, T.J., Kuick, R., Serecky, K., Hammer, G.D., 2009. Wnt/betacatenin signaling in adrenocortical stem/progenitor cells: implications for adrenocortical carcinoma. Ann. Endocrinol. Paris 70, 156.
Knapp, D.W., Waters, D.J., 1997. Naturally occurring cancer in pet dogs: important models for developing improved cancer therapy for humans. Mol. Med. Today 3, 8–11.
Kobayashi, H., Usui, T., Fukata, J., Yoshimasa, T., Oki, Y., Nakao, K., 2000. Mutation analysis of Gsalpha, adrenocorticotropin receptor and p53 genes in Japanese patients with adrenocortical neoplasms: including a case of Gsalpha mutation. Endocr. J. 47, 461–466.
Kool, M.M.J., Galac, S., Spandauw, C.G., Kooistra, H.S., Mol, J.A., 2013. Activating mutations of GNAS in canine cortisol-secreting adrenocortical tumors. J. Vet. Intern. Med. 27, 1486–1492.
Kool, M.M., Galac, S., Kooistra, H.S., Mol, J.A., 2014. Expression of angiogenesis-related genes in canine cortisol-secreting adrenocortical tumors. Domest. Anim. Endocrinol. 47, 73–82.
Kool, M.M.J., Galac, S., van der Helm, N., Spandauw, C.G., Kooistra, H.S., Mol, J.A., 2015a. Expression of somatostatin, dopamine, progesterone and growth hormone receptors in canine cortisol-secreting adrenocortical tumors. Vet. J. http://dx.doi.org/10.1016/j.tvjl.2015.05.014.
Kool, M.M., Galac, S., van der Helm, N., Corradini, S., Kooistra, H.S., Mol, J.A., 2015b. Insulin-like growth factor-phosphatidylinositol 3 kinase signaling in canine cortisol-secreting adrenocortical tumors. J. Vet. Intern. Med. 29, 214–224.
Lalli, E., 2010. Adrenocortical development and cancer: focus on SF-1. J. Mol. Endocrinol. 44, 301–307.
Lalli, E., Doghman, M., Latre de Late, P., El Wakil, A., Mus-Veteau, I., 2013. Beyond steroidogenesis: novel target genes for SF-1 discovered by genomics. Mol. Cell. Endocrinol. 371, 154–159.
Latronico, A.C., Reincke, M., Mendonca, B.B., Arai, K., Mora, P., Allolio, B., Wajchenberg, B.L., Chrousos, G.P., Tsigos, C., 1995. No evidence for oncogenic mutations in the adrenocorticotropin receptor gene in human adrenocortical neoplasms. J. Clin. Endocrinol. Metab. 80, 875–877.
Li, D., Urs, A.N., Allegood, J., Leon, A., Merrill Jr., A.H., Sewer, M.B., 2007. Cyclic AMP-stimulated interaction between steroidogenic factor 1 and diacylglycerol kinase theta facilitates induction of CYP17. Mol. Cell. Biol. 27, 6669–6685.
Libe, R., Mantovani, G., Bondioni, S., Lania, A.G., Pedroni, C., Beck-Peccoz, P., Spada, A., 2005. Mutational analysis of PRKAR1A and Gs(alpha) in sporadic adrenocortical tumors. Exp. Clin. Endocrinol. Diabetes 113, 248–251.
Lumbroso, S., Paris, F., Sultan, C., European Collaborative Study, 2004. Activating Gsalpha mutations: analysis of 113 patients with signs of McCune–Albright syndrome – a European collaborative study. J. Clin. Endocrinol. Metab. 89, 2107–2113.
Lyons, J., Landis, C.A., Harsh, G., Vallar, L., Grunewald, K., Feichtinger, H., Duh, Q.Y., Clark, O.H., Kawasaki, E., Bourne, H.R., 1990. Two G protein oncogenes in human endocrine tumors. Science 249, 655–659.
Mumby, S.M., Kahn, R.A., Manning, D.R., Gilman, A.G., 1986. Antisera of designed specificity for subunits of guanine nucleotide-binding regulatory proteins. Proc. Natl. Acad. Sci. U. S. A. 83, 265–269.
Naan, E.C., Kirpensteijn, J., Dupre, G.P., Galac, S., Radlinsky, M.G., 2013. Innovative approach to laparoscopic adrenalectomy for treatment of unilateral adrenal gland tumors in dogs. Vet. Surg. 42, 710–715.
Nair, R., Teo, W.S., Mittal, V., Swarbrick, A., 2014. ID proteins regulate diverse aspects of cancer progression and provide novel therapeutic opportunities. Mol. Ther. 22, 1407–1415.
Nishihara, E., Amino, N., Maekawa, K., Yoshida, H., Ito, M., Kubota, S., Fukata, S., Miyauchi, A., 2009. Prevalence of TSH receptor and Gsalpha mutations in 45 autonomously functioning thyroid nodules in Japan. Endocr. J. 56, 791–798.
Niswender, C.M., Ishihara, R.W., Judge, L.M., Zhang, C., Shokat, K.M., McKnight, G.S., 2002. Protein engineering of protein kinase A catalytic subunits results in the acquisition of novel inhibitor sensitivity. J. Biol. Chem. 277, 28916–28922.
Nomura, M., Kawabe, K., Matsushita, S., Oka, S., Hatano, O., Harada, N., Nawata, H., Morohashi, K., 1998. Adrenocortical and gonadal expression of the mammalian Ftz-F1 gene encoding Ad4BP/SF-1 is independent of pituitary control. J. Biochem. 124, 217–224.
Paoloni, M., Khanna, C., 2008. Translation of new cancer treatments from pet dogs to humans. Nat. Rev. Cancer Cancer 8, 147–156.
Parker, H.G., Shearin, A.L., Ostrander, E.A., 2010. Man's best friend becomes biology's best in show: genome analyses in the domestic dog. Annu. Rev. Genet. 44, 309–336.
Parker, K.L., Rice, D.A., Lala, D.S., Ikeda, Y., Luo, X., Wong, M., Bakke, M., Zhao, L., Frigeri, C., Hanley, N.A., Stallings, N., Schimmer, B.P., 2002. Steroidogenic factor 1: an essential mediator of endocrine development. Recent Prog. Horm. Res. 57, 19–36.
Piu, F., Del Tredici, A.L., 2010. Inverse agonist activity of steroidogenic factor SF-1. Methods Enzymol. 485, 245–259.
Ranieri, G., Gadaleta, C.D., Patruno, R., Zizzo, N., Daidone, M.G., Hansson, M.G., Paradiso, A., Ribatti, D., 2013. A model of study for human cancer: spontaneous occurring tumors in dogs. Biological features and translation for new anticancer therapies. Crit. Rev. Oncol. Hematol. 88, 187–197.
Ribeiro, T.C., Latronico, A.C., 2012. Insulin-like growth factor system on adrenocortical tumourigenesis. Mol. Cell. Endocrinol. 351, 96–100.
Rothenbuhler, A., Stratakis, C.A., 2010. Clinical and molecular genetics of Carney complex. Best. Pract. Res. Clin. Endocrinol. Metab. 24, 389–399.
Sato, Y., Maekawa, S., Ishii, R., Sanada, M., Morikawa, T., Shiraishi, Y., Yoshida, K., Nagata, Y., Sato-Otsubo, A., Yoshizato, T., Suzuki, H., Shiozawa, Y., Kataoka, K., Kon, A., Aoki, K., Chiba, K., Tanaka, H., Kume, H., Miyano, S., Fukayama, M., Nureki, O., Homma, Y., Ogawa, S., 2014. Recurrent somatic mutations underlie corticotropin-independent Cushing's syndrome. Science 344, 917–920.
Sbiera, S., Schmull, S., Assie, G., Voelker, H.U., Kraus, L., Beyer, M., Ragazzon, B., Beuschlein, F., Willenberg, H.S., Hahner, S., Saeger, W., Bertherat, J., Allolio, B., Fassnacht, M., 2010. High diagnostic and prognostic value of steroidogenic factor-1 expression in adrenal tumors. J. Clin. Endocrinol. Metab. 95, E161–E171.
Schimmer, B.P., White, P.C., 2010. Minireview: steroidogenic factor 1: its roles in differentiation, development, and disease. Mol. Endocrinol. 24, 1322–1337.
Simon, D.P., Hammer, G.D., 2012. Adrenocortical stem and progenitor cells: implications for adrenocortical carcinoma. Mol. Cell. Endocrinol. 351, 2–11.
Tait, C.R., Jones, P.F., 2004. Angiopoietins in tumours: the angiogenic switch. J. Pathol. 204, 1–10.
Turner, H.E., Harris, A.L., Melmed, S., Wass, J.A., 2003. Angiogenesis in endocrine tumors. Endocr. Rev. 24, 600–632.
Urs, A.N., Dammer, E., Sewer, M.B., 2006. Sphingosine regulates the transcription of CYP17 by binding to steroidogenic factor-1. Endocrinology 147, 5249–5258.
van Sluijs, F.J., Sjollema, B.E., Voorhout, G., van den Ingh, T.S., Rijnberk, A., 1995. Results of adrenalectomy in 36 dogs with hyperadrenocorticism caused by adrenocortical tumour. Vet. Q. 17, 113–116.
Vasudev, N.S., Reynolds, A.R., 2014. Anti-angiogenic therapy for cancer: current progress, unresolved questions and future directions. Angiogenesis 17, 471–494.
Walseth, T.F., Zhang, H.J., Olson, L.K., Schroeder, W.A., Robertson, R.P., 1989. Increase in Gs and cyclic AMP generation in HIT cells. Evidence that the 45-kDa alpha-subunit of Gs has greater functional activity than the 52-kDa alpha-subunit. J. Biol. Chem. 264, 21106–21111.

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