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

Sara Galac*

Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 108, 3508 TD Utrecht, The Netherlands

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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.

* Corresponding author.
E-mail address: S.Galac@uu.nl.

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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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