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Mouse models of adrenal tumorigenesis

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Adrenocortical carcinomas (ACCs) are heterogeneous tumors with a poor prognosis. The rarity of this disorder causes a lack of treatment experience and material availability which is necessary to optimize existing treatments and to develop novel therapeutic strategies. Although surgery is still the treatment of choice, adjuvant therapies are urgently needed as the rate of recurrence for these tumors is high. In recent years molecular characterization of surgical tumor specimen has aided in the understanding of disease mechanisms and definition of therapeutic targets also in adrenocortical carcinoma. However, most of the functional properties of potential target molecules are still unpredictable from pure expression and sequence analysis. For functional studies of gene products, mouse models remain to be intensively utilized as an experimental system due to the similarity to humans with respect to genome organization, development and physiology. Here we give an overview on rodent models that have been described to either have adrenocortical tumors as part of their phenotype or have been utilized for therapeutic screens as adrenocortical tumor models.

Introduction

Mouse models exhibit a wide range of possibilities for the investigation of adrenocortical tumorigenesis. Incidental discovery of adrenal tumors in genetically modified animals can provide clues on pathways involved in adrenal tumorigenesis that would not have been predicted on the basis of structural analysis or *in vitro* exploration. Mouse models can also be used to verify functional significance of a given gene for adrenal growth and steroidogenesis *in vivo* through targeted genetic

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modification. Furthermore, high incidence of adrenal tumors in inbred mouse strains can serve as the starting point for genetic approaches to identify the underlying genetic cause. Similarly, chemically or radiation based mutagenesis could be utilized to create informative mouse models, known as forward (phenotype-driven) genetics. Finally, well-defined tumor models have been successfully used for preclinical intervention trials to screen for novel therapeutic approaches. In this overview we will provide an overview on adrenocortical tumor models relevant for either mechanistic studies or preclinical screening approaches.

Mouse models with spontaneous or induced adrenal tumor growth

Cohen and co-worker in 1951 reported the time course of a group of inbred mice of the LAF1 strain upon exposition to the irradiation of an atomic test bomb explosion (Operation Greenhouse). One male animal developed a tumor in the right adrenal cortex while the left adrenal gland was atrophic. Although it remained uncertain whether the incidence of the adrenal tumor was in fact induced by the irradiation or arose spontaneously, necropsy of the mouse revealed numerous small metastases of the lung as proof of the malignant phenotype of this adrenocortical tumor.[1] The tumor was excised and implanted intramuscularly in the thigh of male and female LAF1 from which it was maintained as a transplantable tumor. While metastatic spread was initially noted on a regular basis in host animals upon later passages the tumors lost their metastatic properties.[2] Histological and laboratory evidence of steroid secretion by the tumor was evident including thymic involution and adrenal atrophy, increase in serum sodium and decrease of serum potassium as well as eosinopenia and lymphopenia.[1] Tumor-bearing animals were even utilized to assay for ACTH bioactivity from transplanted corticotroph tumors.[3] Consequently, a clone of steroid producing tumor cells was established by Yasamura and colleagues as a continuous cell line named Y1.[4]

Another early documented case of adrenal tumorigenesis in mice has been reported for the inbred mouse strain CE after surgical gonadectomy.[5] This observation could be reproduced not only in the CE/J strain[6] but also in other inbred mouse strains including DBA/2J,[7] C3H, BALB/c[8] and NU/J animals,[9] while other strains such as C57BL/6J[10] and FVB/N[7] were found to be resistant to tumor formation. As both surgical gonadectomy and xenografting of hCG producing tumors[9] were able to induce adrenocortical tumor growth in the described mouse strains it was suggested that chronic elevation of gonadotropins represents a major determinant of adrenocortical tumorigenesis. Thus, gonadectomy-induced adrenal tumorigenesis in susceptible mouse strains was characterized in more detail: The investigated tumors were of a benign or semi-malignant phenotype as metastasis was usually not present. The occurrence of these tumors, also when transplanted into littermates,[11] was accompanied by morphological changes in hormone responsive organs such as the uterus or the mammary glands indicative of sex steroid production.[12] Parts of the adrenal tumors were described to be reminiscent of seminiferous tubules of the testis or follicular structures similar to that found in the cortex of the ovary.[5] In accordance with early morphological findings, later functional and molecular studies in fact revealed the expression of markers which are otherwise restricted to the gonad including receptors for LH and Mullerian inhibiting substance (MIS) as well as steroidogenic enzymes such as P450cyp17 and P450cyp19.[6, 7, 9] Accordingly, an adaption to the gonad's ability to secret sex steroids was detected.[6] Interestingly, this functional change was also accompanied by a switch in the expression of the transcription factor *Gata6* to that of *Gata4*.[6, 7, 9] *Gata4* has been implicated in the regulation of tissue-specific gene expression and cellular proliferation in the gonad.[13, 14] Moreover, over expression of *Gata4* is sufficient to induce expression of gonadal markers in adrenocortical tumor cells *in vitro*.[15] Thus, these findings provide indirect evidence that induction of *Gata4* expression is linked to the phenotypic shift observed in gonadectomy-induced adrenal tumors (Fig. 1A).

To identify genetic markers that are associated with gonadectomy-induced adrenal tumorigenesis Bernichtein and colleagues performed a genome-wide association study in non-susceptible C57BL/6J and susceptible DBA/2J animals.[16] Linkage analysis identified a major locus on chromosome 8 containing 31 candidate genes. Among these genes *Sfrp1*, a dominant negative regulator of the Wnt signalling pathway which is down-regulated in a number of tumor entities through epigenetic modifications was highlighted as a promising candidate.[16] However, the exact contribution of the genes identified on the basis of genetic association studies remains to be determined. In addition, it has been