Precursor Lesions of the Adrenal Gland

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Key Words

Pheochromocytoma · Adrenocortical adenoma · Adrenocortical carcinoma

Abstract

Objective: To review the existing literature for evidence that adrenocortical and adrenomedullary tumours develop through a multistep process of carcinogenesis. **Results:** In the adrenal cortex hyperplasia and adenomas are frequently observed tumours or tumour-like conditions. In contrast, adrenocortical carcinomas are rare. Based on well-validated histopathological scoring systems, benign and malignant adrenocortical tumours can be separated, although a small subset of tumours remains hard to classify. Although extensive follow-up studies might argue against multistep carcinogenesis, analysis of chromosomal imbalances and gene expression profiling studies in these tumours are inconclusive and could give support for both multistep pathogenesis or de novo genesis of carcinomas. A major limit to most of these studies is the small sample size and the lack of extensive clinical (follow-up) data. In the adrenal medulla, pheochromocytomas (PCC) are the most frequent tumours in adults, with an incidence of 8 per million. They can be divided into benign and malignant PCC, but the distinction can only be made when metastases are present. Arbitrarily, lesions of less than 1 cm in diameter are called hyperplastic, but it should be expected that the majority of these are early lesions and if left in situ would grow to classify as PCC. In contrast to cortical tumours, the frequent 1p and 3q loss as

an early event in tumourigenesis of benign PCC is verified in multiple studies. However, studies in malignant PCC yield divergent results, due to the small numbers analysed. **Conclusion:** Taken together, there appears to be a relationship between cortical and medullary hyperplasia on the one hand and cortical adenomas and PCC on the other. However, whether there is a transition from benign to malignant tumours, both cortical and medullary, remains to be determined.

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Adrenal Cortex

Embryologically, the adrenal cortex is derived from the mesoderm, and is composed of 3 layers, the zona glomerulosa, zona fasciculata and zona reticularis. During development of the adrenal cortex a prominent foetal zone is present which undergoes apoptosis after birth [1]. This foetal cortex produces dihydroepiandrosterone sulphate and cortisol. In vitro studies showed that insulin-like growth factor (IGF) II is highly expressed in the foetal cortex [2]. After remodelling of the adrenal gland the production of hormones is taken over by the definitive adrenal cortex, and altered due to different requirements. Tumours or tumour-like conditions that arise from the adrenal cortex include hyperplasia, which may be diffuse or nodular, adenomas and carcinomas. The distinction between nodular hyperplasia and adenoma is largely based on gross inspection, in which there are usually

multiple nodules in the former condition and a single nodule with atrophic surrounding cortex in the latter. The distinction between adenomas and carcinomas is based upon size and a set of histological criteria, which allows unequivocal diagnosis in the majority of cases, although there remains a group of tumours that is difficult to classify [3–6].

Adenomas and hyperplasia of the adrenal cortex occur in 1–5% of the general population. In contrast, adrenocortical carcinomas (ACC) only occur in 1 per million. Due to advanced imaging techniques, tumours of the adrenal are increasingly found, also in patients analysed for other complaints. These incidentalomas are surgically excised based on their radiological appearance, size, and symptoms of hormonal overproduction. Thus, larger tumours (>5 cm) are usually removed. The remaining smaller tumours, with or without subclinical endocrine alterations, have been studied with long-term follow-up, showing slow enlargement in a subset of these patients and minor subclinical endocrinological changes, but no progression toward clinical malignancy [7–9]. These studies include mainly adult patients. Paediatric adrenocortical tumours mostly arise in the context of (tumour) syndromes, such as Beckwith-Wiedemann syndrome, Li-Fraumeni syndrome, multiple endocrine neoplasia type 1 (MEN1) and Carney complex. The first 2 syndromes are associated with carcinomas, whereas the latter 2 are usually associated with adenomas [10]. An interesting case report by Bernard et al. [11] described an adult male patient with an adrenocortical tumour composed of a benign and a malignant part. The authors hypothesized that the benign component developed into the malignant component, thus supporting the idea of multistep tumorigenesis. However, whereas the malignant component showed multiple genetic abnormalities, no chromosomal aberrations and no molecular changes were observed in the benign lesion. Thus, although suggestive, this particular case cannot be taken as definitive proof of multistep carcinogenesis in adrenocortical tumours.

Molecular Abnormalities in Adrenocortical Tumours

It is generally accepted that tumours are clonal proliferations of cells with genomic instability. This implies that tumours are homogeneous with respect to early genetic aberrations and heterogeneous for later events.

X-chromosomal inactivation studies have been performed on adrenocortical tumours. The general consensus in these studies is that hyperplasia is a polyclonal con-

dition, although 2 studies revealed a monoclonal pattern in 21 and 22%. Adenomas were polyclonal in 17%, but more frequently monoclonal in 82%. All ACC studies in the literature revealed monoclonality (100%, $n = 28$) [12–15].

Comparative genomic hybridization (CGH) is another tool to detect (genome-wide) genetic aberrations. In a study performed by Kjellman et al. [16] genetic aberrations were found in 2 of 14 tumours investigated. These tumours showed 1p loss and 1q gain in one case, and 9q gain in the other tumour. In another CGH study, Zhao et al. [17] found that 2 of 6 hyperplastic lesions showed a single chromosomal gain of chromosome 17q. This chromosomal aberration was also present in several adenomas, together with gain of chromosome 9. In concordance with the CGH study by Kjellman et al. [16], not all adenomas (only 15 of 23) revealed chromosomal changes. In 2 subsequent studies by Dohna et al. [18] and Sidhu et al. [19], using CGH, genetic changes were found in 6 of 8, and 11 of 18 adenomas, respectively. The study by Sidhu et al. [19] showed gain of chromosome 4q in 4/18 tumours as the most frequent aberration. The study by Dohna et al. [18], however, showed numerous (>5) chromosomal aberrations in 4/8 adenomas investigated. Taken together, 36/69 (52%) adrenocortical adenomas showed chromosomal aberrations, indicating a monoclonal lesion. Adding up all chromosomal alterations in the aforementioned studies, the highest frequency of gain was 9q35-ter (9/63, 14%) of all adrenocortical adenomas investigated. The most common loss observed was 1pcen-31 observed in 3 of the 63 tumours (5%). In contrast to the few genetic alterations found in adrenocortical adenomas, all carcinomas exhibited a wide range of aberrations throughout the whole genome, using CGH [16–19]. The highest frequency of gains was found on chromosome 12q (44%), followed by 5p (39%) and 5q (37%). The highest rate of loss, at the telomeric side of chromosome 1p, was 27%, but this could be an underestimation since 1 study did not analyse this part of the chromosome. Taken together, a proportion of adrenocortical adenomas and carcinomas revealed a limited overlap in the pattern of genetic abnormalities in CGH, as shown by loss of chromosome 5p (6% in adenomas, 39% in carcinomas). However, this could also be explained by the wide range of genomic aberrations in ACC, which makes such an overlap coincidental. A proportion of the frequent aberrations was unique to ACC, e.g. 7p gain in 32% of the carcinomas and 4p gain in 23% of the carcinomas. In addition, as described by Dohna et al. [18], carcinomas exhibited a range of amplifications, a feature that is rare in adrenocortical adeno-

Fig. 1. Precursor lesions.

mas. In paediatric adrenocortical tumours described by Figueiredo et al. [20], the most striking aberration was gain and amplification of chromosome 9q34 in 4 carcinomas and 1 adenoma. Interestingly, this was performed on Brazilian patients, who were known to have a high incidence of P53 gene mutations [21]. However, loss of 17p was only found in 1 tumour.

More recently, gene expression profiling has been employed to study adrenocortical tumours, using DNA microarrays [22–24]. In this way, with the use of different DNA microarray platforms, it was shown that adenomas and carcinomas had different gene profiles and clustered separately. One of the main discriminating gene clusters for the separation of adenomas from carcinomas contained the IGF II gene. The up-regulation of this gene is well documented in previous investigations [25]. Increased expression of the IGF II gene cluster was correlated with adverse prognosis [23]. In paediatric adrenocortical tumours the most striking difference between adenomas and carcinomas was observed for the FGFR4 gene, but the carcinomas also displayed overexpression of IGF II, not only with respect to adenomas but also in comparison with normal human foetal adrenal cortex [26]. As suggested in the literature, IGF II could confer growth advantage to adrenocortical tumour cells leading to ACC formation. IGF II is also known to induce adrenal hyperplasia in mice [27] and embryonic stem cell-induced neural cells revealed an increased proliferation rate in culture probably triggered by IGF II [28].

Adrenal Medulla

In the adrenal medulla, tumourigenesis originating from chromaffine cells leads to the formation of pheochromocytomas (PCC), which occur with an incidence of approximately 1–2 per 100,000. Although most PCC are benign, malignancy is found in 10% of these tumours, with up to 50% malignancy if the tumour is located outside the adrenal [29, 30]. The issue of prediction of clinical PCC behaviour has been the topic of many investigations over the past decades [31]. Recent scoring systems, based on the histology of the primary tumour as well as catecholamine measurements, could be of help in the determination of malignant behaviour [32, 33]. However, until now the decisive criterion for malignancy is the presence of histologically proven metastases in places where no chromaffine tissue is present.

Up to 25% of PCC appear to occur in the context of one of several hereditary cancer syndromes. These include MEN2, von Hippel-Lindau disease and the recently discovered PCC-paraganglioma syndrome [34]. Due to the hereditary nature and the elucidation of the causative genes for these syndromes, affected individuals can be detected at an early stage of their disease. This has allowed the study of potential precursor lesions of PCC [35]. Some patients with elevated catecholamine levels only show diffuse or nodular hyperplasia of the adrenal medulla, whereas others have such hyperplasia in combination with a PCC. This led to the idea that hyperplasia

of the adrenal medulla is the precursor of PCC. It should be stated at this point that the distinction between nodular hyperplasia and PCC is purely based on the diameter of the lesions being smaller or greater than 1 cm, respectively. In addition, malignancy in MEN2-related PCC is very rare. In contrast to hereditary PCC, most sporadic PCC do not show hyperplasia of the surrounding or contralateral medulla.

At the molecular level, there seem to be distinct pathways towards PCC formation, with loss of chromosome 1p and 3q being the early events in MEN2-related and sporadic benign PCC [36, 37]. Because the genetic aberrations are similar, it appears that sporadic and at least a proportion of hereditary PCC may arise through the same pathway. However, in von Hippel-Lindau disease-related PCC there are specific losses of 3p and 11p that appear to be early events but 1p loss is rare [38]. The different aberrations could represent different pathways leading to the same tumour type.

In addition to the previously described CGH studies on benign PCC, small series of malignant PCC have been analysed in the literature [36, 37, 39, 40]. Few chromosomal regions were shown to be selectively involved in malignant PCC in contrast to benign PCC, such as gain of 5p and loss of 8p. Also, the presence of more than 10 chromosomal aberrations is found in malignant, but not in benign PCC [40]. Other studies, as well as preliminary data from our group show that gain of chromosome 5p and a high expression of human telomerase reverse transcriptase are linked to malignant behaviour [41–43]. This corresponds with our recent array-CGH findings that show a gain of chromosome 5p in 50% of malignant PCC, and only in 5% of benign PCC [unpubl. observations]. This gain was not only found in metastases but also in primary tumours.

Gene expression profiling to distinguish benign from malignant PCC is only published in summarized form [44]. Here the authors state that there are multiple genes that are differentially expressed in benign and malignant PCC and malignant potential is largely characterized by a less differentiated pattern of gene expression. However, since no gene clusters were defined in this article, the differentiation of benign and malignant tumours based on gene expression profiles needs to be confirmed.

Discussion and Conclusion

Taken together, similar genetic defects in adrenocortical hyperplasia and adenomas might be related to the progression of a hyperplastic lesion towards an adenoma.

Therefore, cortical hyperplasia could be a precursor lesion for an adenoma. For the molecular studies performed on adrenocortical adenomas and carcinomas, the number of tumours investigated is limited. Based on CGH, adenomas have few aberrations and no overlap is present between various studies. The same is true for CGH studies of ACC [16–19]. Therefore, these studies do not allow definitive conclusions on the evolution of adrenocortical adenomas into ACC. However, to disprove the precursor theory the data are also insufficient, as there is no chromosomal region of loss or gain in adenomas that is not affected in carcinomas. From a clinical point of view, adrenocortical tumours that are small, usually classified as incidentalomas, appear to have no clinical tendency towards malignancy in long-term follow-up [7–9]. Although these follow-up studies are based on large numbers of tumours, this does still not constitute definitive evidence against the multistep theory of carcinogenesis, as ACC are extremely rare. In addition, gene expression profiles can differentiate between benign and malignant adrenocortical tumours, with IGF II playing a major role [22–24, 26]. IGF II overexpression is found in both adult and paediatric ACC, and could be a hallmark of all forms of ACC. Still, this is insufficient proof to support that there is a transition from adrenocortical adenomas to ACC.

Precursor lesions in the adrenal medulla appear to exist, as has been suggested for the adrenal cortex, with hyperplasia of the medulla leading to benign PCC in some cases. However, it must be stated that the difference between hyperplasia and PCC is somewhat artificial, as it is purely based on a size criterion. The CGH data from benign PCC are consistent in various studies from various groups, but the genetic abnormalities in malignant PCC have not been entirely elucidated, largely due to the lack of sufficiently large studies adhering strictly to the definition of malignancy for PCC. The most consistent data on malignant PCC is the accumulation of multiple defects, in contrast to the benign PCC [36, 37, 39, 40]. At present, there appears to be insufficient proof that benign PCC can develop into malignant PCC through the accumulation of additional genetic abnormalities. Although preliminary, gene expression profiles suggest that malignant PCC are less differentiated tumours, and differ from benign PCC [44].

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