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Association Involving PKA, the Cellular Cycle, and Spermatogenesis in Sympathetic Nervous Tumor Tissue

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Abstract

Cell-cycle dysregulation and increased steroid production are frequent effects of adrenocortical malignancies. The control of the cell cycle and steroid production are both impacted by cAMP/PKA signalling. We looked into the regulation of steroidogenesis and the role of PKA in adrenocortical cells using cell synchronisation in the various cell-cycle phases. At G2 phase, cells had their highest levels of PKA activity, while at G1 phase, they had their lowest levels. In all synchronised groups, PRKACA overexpression or cAMP stimulation increased PKA activity and promoted steroidogenesis, but these effects alone are insufficient to promote cell-cycle advancement. Only in cells in G1, PRKAR1A inactivation increased PKA activity, promoted STAR gene expression, and prompted cell-cycle advancement in all groups. These results demonstrate a close connection between steroidogenesis and the cell cycle in ACTs. Moreover, PRKAR1A plays a critical role in controlling how PKA activity affects adrenocortical cells' ability to advance through the cell cycle and steroidogenesis.

Keywords: Dysregulation; Steroidogenesis; Adrenocortical cells; Cell cycle

Introduction

Increased steroid secretion is frequently linked to adrenocortical tumours, which increases morbidity. Malignant adrenal cortical carcinoma and benign tumours such macro nodular hyperplasia, adenomas, and primary pigmented nodular adrenocortical disease have all been linked to this. Mass spectrometry study has shown that even ACCs, which are often categorised as endocrine-inactive based on hormonal assessment, can cause aberrant steroid release. Cell-cycle control flaws are another common ACT cell abnormality [1]. This discovery is supported by the fact that ACCs are more likely to exhibit amplifications and deletions of the genes that encode important cell cycle regulators.

Although it is well known that the effects of steroid hormones, such as glucocorticoids, androgens, and oestrogens, on cell proliferation and differentiation are mediated by the regulation of crucial processes in the control of cell-cycle progression or arrest, little is known about steroid hormone secretion during the cell cycle in the adrenal gland [2]. In the course of treating ACC, the adrenolytic medication mitotane reduces cortisol output and causes the adrenal cortex's zona fasciculata and zona reticularis to die. Its use has been linked to apoptosis and cell-cycle arrest at the G2/M phase.

Steroid over-secretion is caused by genetic changes that result in constitutive activation of the cAMP/PKA pathway in different types of ACTs, such as PPNAD or cortisol-secreting adenomas. The greatest examples are inactivating mutations of the regulatory component R1A of PKA, which are found in a subset of secreting ACAs and ACCs as well as tumour DNA and germline DNA of patients with PPNAD and Carney complex. When the relationship between PRKACA and the regulatory subunits of PKA, such as PRKAR1A, is broken down, PRKACA somatic mutations also cause constitutive PKA activity in benign adrenal tumours linked to Cushing syndrome [3]. The cell cycle is regulated by variations in cAMP signal transduction. Many cell types are affected by activation of the cAMP/PKA pathway, which slows proliferation by keeping cells from entering the S phase and stopping them in the G1 phase. On the other hand, activation of the cAMP/PKA pathway may promote cell growth, as in pituitary and thyroid cancers.

Protein kinase A is predominantly activated by cyclic AMP to carry out its functions. Two different types of regulatory and four different types of catalytic subunits make up PKA. The function of the PKA regulatory subunits in the development of tumours has been investigated. Studies conducted in vivo and in vitro have demonstrated that inactivating PRKAR1A causes cancer. These results shown that primary mouse embryonic fibroblasts are constitutively activated by PKA and become immortal when Prkar1a protein is absent from primary mouse cells in vitro [4, 5]. D-type cyclins were upregulated as a result of knocking down Prkar1a at the molecular level. Similarly, we demonstrated that PRKAR1A inactivation increases proliferation and provides resistance to apoptosis in the adrenocortical cell line H295R. Cells accumulated in the G2 phase and cyclin D and PKA CA activity increased as a result of PRKAR1A inactivation disrupting the cell-cycle checkpoint. According to these findings, there might be a connection between the cell-cycle disruption that is concurrently seen in adrenal tumours and the rise in steroid release.

Using two cell lines synchronised by pharmacological drugs, H295R, a human adrenal cortex cell line derived from an ACC that does not harbour any mutations in the cAMP/PKA pathway, and primary cultured PPNAD cells, which have inactivating mutations of PKAR1A, were used to study the cell-cycle-dependent control of adrenal-steroid over secretion and the role of cAMP/PKA. We used cAMP treatment, temporary silencing of PRKAR1A by transfection with a siRNA, or overexpression of one of the catalytic subunits of PKA in conjunction with the pharmacological drugs to mimic the constitutive activation of PKA seen in adrenal tumours with PRKAR1A inactivating or PRKACA activating mutations [6]. Overall, our results indicate that

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the cell cycle phase regulates the hyper secretion of steroids by the H295R ACC cell line and PPNAD and that PKA activity participates in this coordination.

The mitotic cell cycle in the majority of eukaryotic cells contains four phases: G1, S, G2, and M. The cyclin-dependent kinase 1 (Cdk1)/ Cyclin B complex is activated during the G2/M transition. The Wee1 kinase family immediately inhibits Cdk1 linked with Cyclin B during the late S and G2 phases by phosphorylating it on its Thr14 and Tyr15 [7]. The Cdc25 phosphatase family dephosphorylates Cdk1 on both Thr14 and Tyr15 and activates it to enter the M phase.

The Wee1 and Myt1 kinases make up the Wee1 kinase family in metazoans. Whereas the membrane-associated kinase Myt1 phosphorylates Cdk1 on both Thr14 and Tyr15, the nuclear kinase Wee1 only does so on Tyr15. Wee1 has two isoforms in Xenopus: wee1A and wee1B. Wee1A is expressed from mature oocytes to gastrula embryos but not from fully developed immature oocytes. On the other hand, post-gastrula embryos express Wee1B. During both oocyte maturation and early development, Myt1 is continuously expressed. Wee1 is crucial for the inhibitory phosphorylation of Cdk1 during the somatic cell cycle of mammals [8-10]. In Xenopus laevis, starfish, and C. elegans, Myt1 directly contributes to the prophase I arrest of developing oocytes by blocking the Cdk1/Cyclin B complex. Myt1 is not necessary for the normal development of the cell cycle in somatic cells, but it is necessary to prevent an increase in cytotoxicity following checkpoint abrogation brought on by Wee1 inhibition. Moreover, Xenopus laevis has the cdc25 isoforms cdc25A, B, C, and D. Cdc25C is continuously expressed during oocyte maturation and the early stages of development. Cdc25A mRNAs are present in oocytes, but their translation doesn't start until after fertilisation. The expression of the cdc25B and D mRNAs occurs in the zygote as opposed to the mother.

In C. elegans, Drosophila, and Xenopus, the successive cell cycles are short, but the initial cell cycle immediately after fertilisation is protracted. In Xenopus embryos, the first mitotic cell cycle is slow, while the 2–12 cell cycles mostly S and M phases without interfering G1 and G2 phases are relatively quick. Xenopus eggs undergo meiosis after fertilisation and then go through a unique initial mitotic cycle with S, G2, and M phases [11]. Both a male and female pronuclear migrate inward and congress before the first M phase, and the cytoplasmic cortex rotates, which results in the development of dorsal cell fates. As a result, it is believed that the G2 phase of the first cell cycle is crucial for allowing time for these crucial developmental activities.

As Cdk1 is significantly phosphorylated at its inhibitory phosphorylation sites during the G2 phase of the Xenopus first cell cycle but barely at all throughout the 2–12 cycles, it is possible that different mechanisms regulate Cdk1 phosphorylation before and after the first cell cycle. Inhibitory phosphorylation of Cdk1 increases when the Cdc25A protein is expressed in cleavage-stage embryos, delaying cell division and indicating that Cdc25A is engaged in the fast cell cycle at this time [12, 13]. Furthermore, suppressing Wee1A activity with a dominant negative version shortens the G2 phase of the first cell cycle in Xenopus eggs and reduces inhibitory phosphorylation of Cdk1. The inhibitory phosphorylation of Cdk1 is severely suppressed by PD0166285, a pharmacological inhibitor of both Wee1 and Myt1 kinases, which also shortens the G2 phase in the Xenopus first cell cycle. These earlier investigations imply that the length of the first cell cycle may be controlled by a balance between Cdc25A, Wee1A, and Myt1. Yet, it is still unclear how this equilibrium is maintained during the first cell cycle of Xenopus laevis. Particularly, no research has been done into Myt1's function throughout this time.

In this study, we demonstrated that the amount of Cdc25A present in Xenopus eggs did not significantly affect the length of the first cycle. We achieved this by employing an antisense morpholino oligonucleotide against cdc25A mRNA and a stable Cdc25A mutant [14]. As opposed to Wee1A inhibition using an antisense morpholino oligonucleotide, Myt1 inhibition with a neutralising antibody significantly decreased inhibitory phosphorylation of Cdk1 throughout the first cell cycle. Our findings imply that Myt1 and not Wee1A is primarily in charge of determining how long the first cell cycle's extended G2 phase lasts.

Methods

In DMEM Ham/F12 media supplemented with 50 units/ml penicillin, 50 mg/ml streptomycin, 2 mmol/L glutamine, 2% Ultroser G2, and ITS at 37°C in an atmosphere of 5% carbon dioxide/95% air, human H295R adrenocortical carcinoma cells were cultured as previously described [15]. By giving the cells treatments with L-mimosine, aphidicolin, or nocodazole, we synchronised the cells, which were then examined by cytometry analysis and propidium iodide staining. Both cell types produced findings that were comparable. The profile of asynchronous cells treated with vehicle was normal, with a larger proportion of cells in G1. L-mimosine treatment caused an increase in G1 cells and a decrease in G2 cells as a percentage of total cells.

Discussion and Conclusion

Mutations in a number of crucial cAMP pathway elements, which are also probably implicated in the development of adrenal tumours, can explain excessive adrenal-steroid output. Here, we show that steroidogenesis is controlled by particular cell-cycle phases in both a primary cell culture of PPNAD and an established human adrenal cortical cancer cell line, H295R. In this investigation, we discovered that Myt1 inhibition had a higher effect than Wee1A inhibition on the degree of Cdk1 phosphorylation in the first cell cycle. Our findings imply that Myt1 is primarily involved in this Cdk1 phosphorylation, which results in a prolonged G2 phase during this time. Recent research has established a direct role for Myt1 in the arrest of Xenopus immature oocytes in the G2 phase. Our findings showed that Myt1 played a second significant role in Xenopus development.

Declaration of Competing Interest

There is no conflict of interest.

Acknowledgements

The author has well explained the entire topic.

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