Caldesmon, an actin-linked regulatory protein, comes across glucocorticoids

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The glucocorticoids (GCs), the most downstream effectors of the hypothalamic-pituitary-adrenal (HPA) axis, are the main mediators of stress response. Stress-triggered GCs as well as acute and chronic GC treatment can impair the structural plasticity and function of the brain. The exposure of perinatal animals and humans to excess stress or GCs can affect the brain development, resulting in altered behaviors in the adult offspring of animals and an increased risk of psychiatric disorders in humans. Despite the numerous studies documenting these effects, the underlying mechanism remains unclear. In this commentary we will focus on the effect of excess GCs on cortical development. We have recently showed that excess-GC-dependent retardation of the radial migration of neural progenitor cells (NPCs) is caused by the dysregulation of actin-myosin interaction via upregulation of caldesmon (CaD), an actin-linked regulatory protein. The elucidation of the molecular mechanisms that underlie the detrimental action of GCs on cortical development will expand our understanding of how stress/GCs alter the formation of neural networks and affect behaviors later in life.

It has been well documented that the elevation of GCs in response to stress impairs the development and function of the brain.1,3 Prenatal stress is associated with an increase in abnormal behaviors of adult offspring.2 In humans, maternal stress increases the risk of psychiatric disorders, including anxiety, depression and schizophrenia, in the offspring during adolescence and adulthood.3,4 The treatment of neonatal rats with GC transiently retards their brain development,3,5,7 and giving GC to pregnant sheep retards fetal brain growth.8 Antenatal exposure to synthetic GC in humans results in a reduced cortex convolution index and surface area in infants.9 Thus, excessive stress or GC exposure during the perinatal stages affects the brain development and subsequently causes abnormal behaviors in experimental animals and an increased incidence of psychiatric disorders in humans. Despite the numerous studies documenting these detrimental effects of GCs, the underlying mechanism remains unclear.

Cortical development progresses by two types of neuronal migration: radial and tangential.10,11 Postmitotic NPCs in the ventricular zone (VZ) migrate radially along radial glial fibers toward the surface of the neocortex, until they reach their final destination within the cortical plate (CP). About 80–90% of all cortical neurons arise from NPCs by radial migration. The remaining cells, which include the majority of GABAergic interneurons, migrate tangentially from the ganglionic eminence to the neocortex. Several human disorders that arise from defective neuronal migration have been identified. These disorders include periventricular nodular heterotopia and lissencephaly, which are caused by mutations in the genes involved in radial migration.12 More commonly than gene mutations, however, environmental factors such as stress-triggered GCs as described above are implicated in inducing abnormalities in brain development. Most recently, we have found that excess GCs cause a change in radial migration during cortical development via...
the dysregulation of actomyosin system by GC-induced upregulation of CaD.13

**Detrimental Effects of Excess GCs on Cortical Development and Identification of Target for GCs**

As described in the introduction, the elevated levels of stress-triggered GCs impair the development and function of the brain. To elucidate the underlying mechanism during cortical development, we investigated the effect of excess GC on radial migration. The treatment of pregnant rats with excess GC during the last week of gestation transiently retarded the radial migration of bromodeoxyuridine (BrdU)-labeled NPCs at the boundary between the intermediate/subventricular zone (IZ/SVZ) and the CP, throughout perinatal corticogenesis.13 Using a culture system of NPCs, we examined the direct effect of GC on NPC migration. GC treatment caused changes in the NPCs’ morphology, from a bipolar to multipolar shape, and in their migratory path, which shifted from travel in a single direction to random wandering. In addition, the tips of multipolar processes in the GC-treated NPCs appeared to have dynamic neurite growth endings13 (Fig. 1A). The same phenotypic changes were found in cells located within the IZ of GC-treated embryonic brains in vivo.13

To identify the genes that induce these phenotypic changes, we sequentially screened for GC-responsive genes using microarray analysis, real-time qPCR, and western blotting, selected the GC-responsive genes that encoded cytoskeletal proteins, and identified the **CALD1** gene encoding CaD as the sole target. A half-maximal increase in CaD expression was achieved by treatment with about 200 nM corticosterone (CORT),13 which corresponds to the circulating levels of rodent CORT when under severe stress. Thus, pathological levels of GC can upregulate CaD expression.

The in vivo knockdown of endogenous CaD in NPCs (by in utero electroporation of CaD microRNAs) markedly impaired radial migration, suggesting that CaD is important for radial migration in vivo.13 Furthermore, the forced expression of GFP-CaD in NPCs by in utero electroporation transiently retarded radial migration at the boundary between the IZ/SVZ and the CP, throughout cortical development.13 Thus, an appropriate level of CaD expression is critical for radial migration of NPCs, and CaD overexpression precisely mimics the GC-dependent retardation of radial migration of NPCs as revealed by BrdU labeling.

**GC-Dependent Transcription of the **CALD1** Gene Encoding CaD**

CaD was originally identified as an actin- and calmodulin-binding protein.14 Two isoforms with different molecular weights (Mr), high Mr CaD (h-CaD) and low Mr CaD (l-CaD), are generated from a single gene by alternative splicing.15 They have identical N- and C-terminal domains, but differ by the specific insertion of a central repeating domain in h-CaD. Both isoforms inhibit the actin-myosin interaction, and Ca2+/calmodulin reverses this inhibition. This regulatory function of CaD maps to its C-terminal domain, which has actin-, calmodulin- and tropomyosin-binding activities.16 CaD can also stabilize actin filaments. Thus, CaD is a regulator of the actin-myosin interaction and a potent stabilizer of actin filaments.16 h-CaD is specifically expressed in smooth muscle cells (SMCs), and l-CaD is ubiquitously distributed in non-muscle cells, including neurons and NPCs.16 We refer to l-CaD as CaD in this commentary.

Castellino et al. reported that GCs induce the upregulation of CaD at the protein and mRNA levels and the CaD-dependent reorganization of actin filaments in some cell lines.17 However, the mechanism underlying the GC-induced upregulation of CaD remained unknown for a decade. It has been demonstrated that the transcription of the **CALD1** gene encoding CaD in SMCs and non-muscle cells is dependent on serum response factor (SRF).18,19 Myocardin is demonstrated as a co-factor for SRF-mediated transcription of the cardiac- and SMC-restricted genes and is considered a crucial regulator of their differentiation.20,21 In non-muscle cells, myocardin-related transcription factors (MRTFs) transactivate the actin cytoskeletal genes, including the **CALD1** gene, via the Rho-MRTF-SRF pathway.19 We demonstrated that in NPCs, GC upregulated CaD expression mediated by glucocorticoid receptors (GRs). An activated form of GR directly bound to the two GC-response elements (GREs) located in the **CALD1** promoter, and the **CALD1** gene was subsequently transcribed.13 The Rho/MRTF/SRF pathway was not required for the GC-dependent transcription. The identical, GR-dependent transcription of the **CALD1** gene through the GRE sequences may be a common mechanism among mammalian species.

**Involvement of CaD in Neuronal Migration**

Migration of postmitotic NPCs (neuronal migration), such as radial and tangential migration, is a central feature of cortical development.10,21 Migrating cells exhibit a bipolar shape with a characteristic leading process that extends over long distances and the cell rear that retracts in response to migration. Directions of neuronal migration must be coupled to nuclear movement and centrosomal locomotion.23,24 It has been demonstrated that actin and myosin II interactions are involved in the movement of nucleus and centrosome in connection with neuronal migration. Schaar and McConnell propose an antagonistic relationship between microtubules and myosin II-mediated contractility during neuronal migration. An absence of microtubules at the cell rear triggers myosin II-mediated contraction, which generates a pushing force on the nucleus.25 In contrast, Solecki et al. show that myosin II and F-actin enriched in leading process pull the centrosome and soma, including the nucleus, forward during glial-guided migration via the conserved polarity protein Par 6a.26

We examined the localization of CaD, F-actin and myosin II in cultured NPCs. Both CaD and F-actin were mainly co-localized in the soma and the tip of leading process. Myosin IIA and IIB are the most prominent myosin II isoforms expressed in neurons. In migrating NPCs, myosin IIA was localized in the soma in addition to the tip of leading process and myosin
In GC-treated cells, CaD, F-actin and myosin IIA were localized in the soma and concentrated in the neurite growth endings at the tips of multipolar processes. Myosin II B was localized in the soma, not concentrated in the tips of multipolar processes. Similar to the effects of GC treatment, GFP-CaD-overexpressing cells exhibited a bipolar-to-multipolar transition with the dynamic neurite growth endings at the tips of multipolar processes and migrated randomly (Fig. 1B). As mentioned previously, the C-terminal domain of CaD (C-CaD) inhibits interactions between actin and myosin. The expression of exogenous C-terminal GFP-CaD (GFP-C-CaD), but not of N-terminal GFP-CaD (GFP-N-CaD), also induced the same phenotypic changes as GC treatment and GFP-CaD overexpression. Treatment with blebbistatin, a selective inhibitor of myosin II ATPase, or depletion of myosin IIA and/or myosin IIB by their small interfering RNAs (siRNAs) also induced these phenotypic changes (Fig. 1C and D). These data indicate that an appropriate level of CaD expression is critically involved in a bipolar shape of NPCs with linear migration via the control of action-myosin II interaction. However, GC-induced overexpression of CaD affects the cell shape and migration of NPCs by inhibiting actin-myosin interaction, rather than by stabilizing actin filaments. Why do the inhibition of actin-myosin II interaction by CaD overexpression or by the downregulation of myosin IIA/B functions with blebbistatin treatment or their depletion induce such phenotypic changes of NPCs? It has been demonstrated that similar to neuronal migration, myosin II in fibroblasts plays a prominent role for front-back polarity and nuclear and centrosomal orientation during polarized migration. Indeed, these cells depleting or inhibiting myosin II exhibit multiple protrusions, loss of front-back polarity, and mislocalization of nucleus and centrosome. Taken together, we consider that GC-induced upregulation of CaD in migrating NPCs inhibits interactions between myosin II and perinuclear actin filaments that directly link to the nucleus and centrosome, leading to bipolar-to-multipolar transition of NPCs with random migration via predominance of microtubules.

Recent studies demonstrate that during cortical development, migrating cells exhibit a multipolar shape within the IZ/SVZ, and then undergo a multipolar-to-bipolar transition just before migrating radially from the IZ/SVZ to the CP. Cytoskeletal proteins, including LIS1 and doublecortin, are implicated in this multipolar-to-bipolar transition, and their mutations cause periventricular nodular heterotopia and lisencephaly. In addition, Cdk5 phosphorylates a number of cytoskeletal proteins and is required for the multipolar-to-bipolar transition, as shown in mice with a cortex-specific conditional knockout of Cdk5. The molecular mechanisms of multipolar-to-bipolar transition involving LIS1, doublecortin and...
Cdks remain, however, unclear. Although the loss or gain of these proteins’ functions impair both radial migration and the multipolar-to-bipolar transition in the developing cortex, none of them respond to CGs. Therefore, our findings indicate that CaD is the prime target of GC in the GC-induced abnormality of radial migration in vivo. As summarized in Figure 2, the upregulated CaD-linked negative regulation of actin and myosin II interactions in the IZ/SVZ delays the multipolar-to-bipolar transition in vivo, resulting in the transient retardation of radial migration at the boundary between the IZ/SVZ and the CP during cortical development.

Concluding Remarks

Excessive stress/GC exposure to pregnant animals impairs brain development and results in aberrant behaviors in the adult offspring.1–3,5–7,8 Likewise, excessive GC exposure triggered by stress or medications during human pregnancy is implicated in impaired brain development and an increased risk of psychiatric disorders.3–6–9 However, the underlying mechanism of the stress/GC-induced persistent changes in the brain structure and function is unclear except for the epigenetic regulation of GR and BDNF expression.36 Our work reveals the detrimental effects of GCs on cortical development via the dysregulated expression of CaD by GCs. CaD may also play a crucial role as an actin-linked regulatory protein in synaptogenesis, synaptic plasticity, axon outgrowth, and dendritic arborization. Our findings on CaD’s role in the effects of GCs on cerebral development may provide an explanation for cases of abnormal neural network formation and altered behaviors in adult animals exposed to perinatal stress, as well as the increased risk of psychiatric disorders in humans affected by perinatal stress/GC exposure (Fig. 2). Future studies exploring these links will provide important advances in our understanding of brain development and psychiatric disorders.

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Figure 2. Schematic representation of the mechanism underlying the excess GC-induced retardation of radial migration. NPCs generated in the VZ migrate radially along radial glial fibers toward the surface of the neocortex until they reach their final destination within the CP. During this process, migrating cells with a multipolar shape within the IZ/SVZ undergo transition from multipolar to bipolar just before migrating into the CP. In the neocortex treated with GC, the GC-dependent upregulation of CaD inhibits interactions between actin and myosin II in the radially migrating cells, leading to a delay in the cells’ multipolar-to-bipolar transition. This in turn causes a transient retardation of radial migration at the boundary between the IZ/SVZ and the CP. This impairment in radial migration may be a critical contributor to the slowing of cortical development, followed by the abnormal neural network formation, altered behaviors and increased risk of psychiatric disorders.
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