



**Karolinska
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Institutionen för Neurovetenskap

Alterations in proliferation and differentiation of neural stem cells induced by adverse neurodevelopmental factors

AKADEMISK AVHANDLING

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av

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SUMMARY

Predisposition to diseases can be acquired during early stages of development and resolve into an actual disorder later in life. Early life programming defines the association between challenges during pregnancy that result in altered fetal growth, and developmental and adult disorders. This thesis aimed at studying the effects on neurogenesis triggered by a deranged milieu induced by neurodevelopmental insults, namely excess glucocorticoids (GC) or methylmercury (MeHg). We investigated the effects of exposure to the synthetic GC analog dexamethasone (Dex) on proliferation and differentiation of human progenitor cells (hNPC) grown as neurospheres. We found that Dex decreases hNPC proliferation and differentiation by up-regulating DKK1, a known inhibitor of the canonical Wnt signaling, via a glucocorticoid receptor (GR)-mediated mechanism. We then focused on the effects of Dex in rat cortical neural stem cells (NSCs) and found that exposed cells exhibited a decreased proliferation, increased expression of senescence markers, a down-regulation of mitochondrial genes and global DNA hypomethylation associated with a down-regulation of DNA methyltransferases (Dnmt) 1 and 3a. These effects were heritable, being present also in “daughter” NSCs never directly exposed to Dex. Global DNA hypomethylation was also found in the cortex of 3 day-old mouse pups that were exposed to Dex *in utero*. We used the same experimental design to investigate the effects of the environmental contaminant MeHg (at nanomolar concentrations) on NSCs. MeHg had no effect on cell viability, but reduced the proliferation rate and, similarly to Dex, induced a senescence phenotype associated with down-regulation of mitochondrial genes and global DNA hypomethylation. These changes were also detected in “daughter” NSCs that were never directly exposed to MeHg. Long-lasting effects on NSCs proliferation were also observed in the hippocampal subgranular zone of adult mice exposed to low doses of MeHg during development. The reduced proliferation had a measurable impact on the total number of neurons in the hippocampal dentate gyrus and it could be reversed by treatment with the antidepressant fluoxetine. We further studied the programming effects of GC by genome-wide analysis of differentially methylated DNA regions (DMRs) in NSCs. DMRs occurred in the promoter regions of 575 genes as compared to 1479 in control cells. We selected genes identified as DMR-enriched and found that *Dkk1*, *Dkk3*, *Txnip* and *Cyba* were up-regulated in Dex-exposed proliferating NSCs, and that the changes persisted in daughter cells. We found that the Dex-induced DNA hypomethylation was associated with an up-regulation of Tet1-3 factors and a down-regulation of *Dnmt3a* in both NSCs and postnatal mouse cortex. In Dex-exposed NSCs the expression of *Dkk1* was up-regulated by promoter demethylation in a Tet3-dependent fashion. These effects were also heritable. The Dex-mediated down-regulation of *Dnmt3a* was also dependent on Tet3 expression. In conclusion, our studies show that epigenetic modifications play a critical role in the reprogramming effects exerted by neurodevelopmental insults, such as exposure to excess GC or MeHg.

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