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DISSERTATION

"IL-6 enhances the production of PDGF responsive multipotential progenitors at the expense of neural stem cells - implications for neuropsychiatric disorders"

by

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Cancer Center, G1196

ABSTRACT

A number of studies have highlighted the connection between infections during pregnancy in mothers and increased risk for neuropsychiatric disorders in infants leading to the view that maternal immune activation is a significant contributor to psychiatric illnesses. Cytokines produced maternally can cross the placenta to affect the developing brain. However, it is not clear how these cytokines alter the trajectory of neural development. In my studies on the effects of inflammation on the cells of the subventricular zone (SVZ), I have established that one of the key cytokines implicated in Autism spectrum disorder (ASD), interleukin-6 (IL-6), exerts direct effects on the resident neural stem cells (NSCs) and progenitors. Using neurospheres to model the SVZ, I have found that the IL-6 receptor and GP130 are expressed and that 30 minutes following addition of 5 ng/mL IL-6, STAT-3 phosphorylation increased 7-fold, whereas STAT1, ERK and AKT were unchanged. IL-6 treatment, increased the proportion of PDGF/FGF-2 responsive multipotent progenitors (PFMPs) with a decrease in the proportion of NSCs. Using EdU pulse-labeling in combination with a 4-color flow cytometry panel, IL-6 stimulated a 2-fold increase in EdU incorporation into the PFMPs. This enhanced PFMP proliferation correlated with increased expression of the transcription factors Ascl1 and Gsx1. IL-6 treatment also increased the proportion of neurospheres that were tripotential. To study the effects of systemically increased IL-6 on SVZ cells and brain development, I administered 75 ng IL-6 systemically, which increased the circulating concentration of IL-6 by 3-fold, similar to that documented for ASD patients. When postnatal day 4 male mice were administered 3 doses of IL-6 every 12 hours, the proportion of EdU+ PFMPs was increased 2-fold over controls validating the *in vitro* studies that showed that IL-6 increased PFMP proliferation.

Using RNAseq I have characterized the distinctive molecular fingerprint of the PFMPs as well as several other SVZ neural progenitors. Interestingly, in comparison to SVZ NSCs, PFMPs express high levels of the transcription factor Gsx1, which has been shown to play important role in regulating interneuron development in the forebrain. Also, PFMPs highly express PDGFRα, CSPG4 (NG2+) and transcripts for olfactory receptors. A GO term enrichment analysis of the PFMPs revealed strong expression of genes associated with neurogenesis and gliogenesis, that included activated Notch signaling. In contrast, compared to NSCs, PFMPs expressed lower levels of genes involved in cilia formation, consistent with the loss of cilia as the NSCs become multipotential progenitors.

Next, I investigated the effects of elevated IL-6 levels on the PFMP transcriptome using single cell RNAseq. Differential gene expression analysis of IL-6 treated PFMPS revealed that 387 genes were induced and 248 genes were depressed. Among the top 20 genes that were differentially expressed in IL-6 treated PFMPs vs controls, Fut9 expression was decreased, which is required for expression of Lex, a marker of NSCs and primitive neural progenitors. IL-6 also increased Malat1, a non-coding RNA in PFMPs, which has been shown to affect synaptogenesis. In addition, it also increased HSF1 transcription, a heat shock protein activated in response to environmental stress which increases susceptibility to neuropsychiatric dysfunction. Furthermore, IL-6 downregulated PTEN gene transcription in the PFMPs, which has been shown to be involved in neuropsychiatric disorders.

I also investigated the effects of perinatally elevating levels of IL-6 on behavior when the mice were assessed as young adults. My data show that systemically increasing IL-6 from P3-P5, increased their interactions with a novel mouse in the three-chamber test, which was contrary to my expectation and may suggest that these mice were more aggressive. My data also suggest that they were highly mobile compared to control mice. Systemically increasing perinatal levels of IL-6 in mice increased their anxiety behavior as revealed using the elevated plus maze. Moreover, it also affected their memory consolidation shown by using the inhibitory avoidance test. While short-term memory consolidation was not affected, long- term memory consolidation was affected. These studies show that IL-6 alters the composition of the mammalian SVZ to favor the production of a specific multipotential progenitor, the PFMP, and they reveal molecular changes that accompany IL-6 stimulation of this progenitor. My studies also demonstrate that a short period of inflammation during a critical window of development can have long lasting effects on a range of behaviors that include aggression, anxiety and learning and memory. Altogether, these data provide new insights into the cellular underpinnings of neurodevelopmental disorders associated with maternal infections.