EPIGENETIC PROGRAMMING
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EPIGENETICS

• What is it?
  • Environmentally mediated changes in gene expression
  • Caused by chemical alterations of chromatin and histone structure

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EPIGENETICS

- How do these mechanisms work?
  - Methylation – tightens, inhibits txn
  - Acetylation – loosens, facilitates txn

INTRODUCTION

- Maternal behavior in the rat alters hypothalamic-pituitary-adrenal (HPA) responses to stress
  - High Licking Grooming-Arched Back Nursing (LG-ABN) pups show more modest stress response than Low LG-ABN pups
    - Fear response
  - Cross fostering studies show pups exhibit behaviors of foster parents
INTRODUCTION

- Maternal behavior in the rat alters HPA responses to stress
  - Measured by release of corticotropin-releasing factor (CRF)
  - Magnitude of response mediated by effects on gene expression
    - High: increased glucocorticoid receptor (GR) expression and more mild CRF expression

![Diagram of stress response and GR expression](image)

• Glucocorticoid receptor
  - Increased expression correlated with expression of nerve growth factor-inducible protein A (NGFI-A)
  - Promoter 1 of non-coding exon 1 contains binding site for NGFI-A
    - Higher expression in high LG-ABN groups
Hypothesis: Maternal care alters DNA methylation of the GR exon 1, promoter, and that these changes are stably maintained into adulthood and associated with differences in GR expression and HPA responses to stress.

Figure 1

HOW IS ‘ENVIRONMENTAL PROGRAMMING’ ESTABLISHED AND SUSTAINED IN THE OFFSPRING?

CpG Islands, site of methylation
NGFI-A Binding Site
HOW IS ‘ENVIRONMENTAL PROGRAMMING’ ESTABLISHED AND SUSTAINED IN THE OFFSPRING?

How does CpG methylation in the 17 promoter differ in high and low LG-ABN groups?

Figure 1

Exon 17 of low LG-ABN offspring is more methylated than of high LG-ABN offspring.

How is there a causal relationship between maternal behavior and DNA methylation of offspring?

Cross fostering shows that the maternal behavior determines methylation levels of exon 17 of the GR gene.

Figure 1
How is ‘environmental programming’ established and sustained in the offspring?

Knowing that maternal care only differs in the first week between high and low LG-ABN offspring, does this week correspond to the appearance of differing levels of DNA methylation?

Differences in methylation correspond to timing of differential maternal care.

HOW IS ‘ENVIRONMENTAL PROGRAMMING’ ESTABLISHED AND SUSTAINED IN THE OFFSPRING?

What is the functional importance of these differences in methylation?

What is ChIP?
What is the functional importance of these differences in methylation?

Lower levels of methylation correspond to increased acetylation of H3K9 and NGFI-A binding.

How is ‘environmental programming’ established and sustained in the offspring?

Is the impact of early experience reversible and is epigenetic programming modifiable in adult tissues?

Trichostatin A (TSA) is a histone deacetylase inhibitor.

Treating low LG-ABN offspring with TSA increases acetylation of H3K9
Is the impact of early experience reversible and is epigenetic programming modifiable in adult tissues?

Treatment of low LG-ABN offspring genome with TSA causes demethylation and subsequent acetylation of genomic structures, increasing NGFI-A binding to GR gene promoter.
Is the impact of early experience reversible and is epigenetic programming modifiable in adult tissues?

Methylation of the first 15 CpG dinucleotides is decreased in Low LG-ABN rats treated with TSA.

Figure 4

Methylation of the 5’ and 3’ CpG dinucleotides of the NGFI-A binding region is also decreased in low LG-ABN rats treated with TSA.

Figure 4
HOW IS ‘ENVIRONMENTAL PROGRAMMING’ ESTABLISHED AND SUSTAINED IN THE OFFSPRING?

Does TSA-mediated reversal of epigenetic modifications alter GR expression?

TSA treated low LG-ABN rats show GR expression comparable to High LG-ABN groups.

Figure 5

HOW IS ‘ENVIRONMENTAL PROGRAMMING’ ESTABLISHED AND SUSTAINED IN THE OFFSPRING?

Does TSA treatment alter the adrenocortical response to stress in treated animals?

TSA treated low LG-ABN rats show markedly decreased corticosterone responses as compared to vehicle.

Figure 5
HOW IS ‘ENVIRONMENTAL PROGRAMMING’ ESTABLISHED AND SUSTAINED IN THE OFFSPRING?

- Test 1: Exon 1 of low LG-ABN offspring is more methylated than of high LG-ABN offspring
- Test 2: Cross fostering shows that the maternal behavior determines methylation levels of exon 1 of the GR gene
- Test 3: Differences in methylation correspond to timing of differential maternal care
- Test 4: Lower levels of methylation corresponds to increased acetylation of H3K9 and NGFI-A binding
- Test 5: Treatment of low LG-ABN offspring with TSA causes demethylation and subsequent acetylation of genomic structures, increasing NGFI-A binding to GR gene promoter
- Test 6: TSA treated low LG-ABN rats show GR expression comparable to High LG-ABN groups.
- Test 7: TSA treated low LG-ABN rats show markedly decreased corticosterone responses as compared to vehicle.

SIGNIFICANCE

- Relatability to humans
  - The impact of stressful environments on young people
  - Pharmacological treatment to reverse detrimental epigenetic changes
    - Children of smokers study