La metiltranferasa G9a: del tractament de la Malaltia d’Alzheimer a l'herència de la memòria.

Dr. Christian Griñán-Ferré
Seminari de Recerca
26 de Maig 2022
**Proof-of-Concept (PoC)**

UNC0642 an *in vivo* BBB penetrant G9a inhibitor $IC_{50} < 2.5$ nM

Inhibition of EHMT1/2 rescues synaptic and cognitive functions for Alzheimer’s disease

Yan Zheng,1,2,e Aiyi Liu,1,3,b Zi-Jun Wang,1,4,e Qing Cao,1 Wei Wang,1 Lin Lin,1 Kaijie Ma,1,4 Freddy Zhang,1 Jing Wei,1,4 Emmanuel Matas,1 Jia Cheng,1 Guo-Jun Chen,1 Xiaomin Wang2 and Zhen Yan1,4

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Pharmacological inhibition of G9a/GLP restores cognition and reduces oxidative stress, neuroinflammation and β-Amyloid plaques in an early-onset Alzheimer’s disease mouse model

Christian Griján-Ferré1, Laura Marsal-García1, Aina Bellver-Sanchis1, Shukkoor Muhammed Kondengaden2, Ravi Chakra Turga3, Santiago Vázquez4, Mercè Pallàs1

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Final results
C. elegans study in Budapest
Epigenetics is the study of how your behaviors and environment can cause changes that affect the way your genes work. Unlike genetic changes, epigenetic changes are reversible and do not change your DNA sequence, but they can change how your body reads a DNA sequence.
Histone modifications can alter gene expression.

Heterochromatin is a region of highly compacted DNA that is largely repressive for gene expression.

Histone tails can be modified to change the chromatin state.
Histone modifications can alter **gene expression**.

**Heterochromatin** is a region of highly compacted DNA that is largely repressive for gene expression.

**Histone tails** can be modified to change the chromatin state.
**HISTONE MODIFICATIONS**

**Readers:** Bromo and chromodomains...

**Writers:** Histone methyltransferases and histone acetyltransferases

**Erasers:** Histone deacetylases and demethylases
The human brain expresses numerous genes; approximately 80–95% of genes are expressed.

Neuronal activity *per se* modifies DNA methylation and histone modifications patterns, and further, that learning and memory depend on these epigenetic changes.

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The most common cause of dementia.

A progressive and irreversible age-dependent neurodegenerative diseases characterized by cognitive decline and memory loss.
EPIGENETIC ALTERATIONS ASSOCIATED WITH ALZHEIMER’S DISEASE

INTRODUCTION

↑ H3 and H4 ac
↓ H3K9me2/3
↓ HDAC
↑ H3K4me3
↓ H3K27me3
↑ H3K27me3
↑ miR-34a, miR-132, miR-485
↓ miR-125b, miR-182
↓/↑TET1
↑ DNMT
↓/↑DNMT3a
↑ DNMT1

Gene transcription

Bdnf, Homer1a, Npas-4, Arc, Egr1, c-Fos, zif268

Learning and memory formation

Dendritic spine number and synapse formation

5-hmC
5-mC

Environmental stimuli

Cognitive functions associated with aging

G9a/GLP
ACSS2
↓ H3K9me2/3
↓ H3K27me3

HDAC3
HDAC2
HDAC4
HDAC1

↓ HMT

Bellver-Sanchis et al., 2021. Epigenomes, MDPI.
**G9a METHYLTRANSFERASE**

Lysine methyltransferase.

H3K9me and H3K9me2 are repressive marks.

Its inhibition restores the neuropathological hallmarks of AD.

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*Inhibition of EHMT1/2 rescues synaptic and cognitive functions for Alzheimer’s disease*

Yan Zheng,1,2, a Aiyi Liu,1,2, b Zi-Jun Wang,1,2, a Qing Cao,1, b Wei Wang,1, b Lin Lin,1, b Kaijie Ma,1, b Freddy Zhang,1, b Jing Wei,1,2, c Emmanuel Matas,1, c Jia Cheng,1, c Guo-Jun Chen,1, c Xiaomin Wang2 and Zhen Yan1,2

Epigenetic regulation by G9a/GLP complex ameliorates amyloid-beta 1-42 induced deficits in long-term plasticity and synaptic tagging/capture in hippocampal pyramidal neurons

Mahima Sharma1,2, Tobias Dierkes1,3,4, and Sreedharan Sajikumar1,2

Epigenetics and memory: Emerging role of histone lysine methyltransferase G9a/GLP complex as bidirectional regulator of synaptic plasticity

Karen Ka Lam Panga,b, Mahima Sharmaa,b,c, Sreedharan Sajikumara,b,s

Pharmacological inhibition of G9a/GLP restores cognition and reduces oxidative stress, neuroinflammation and β-Amyloid plaques in an early-onset Alzheimer’s disease mouse model

Christian Griñán-Ferré1, Laura Marsal-García1, Aina Bellver-Sanchis1, Shukkoor Muhammed Kondengaden2, Ravi Chakra Turga3, Santiago Vázquez4, Mercè Pallás1

2017

2019

2019 – Second study in vivo in AD transgenic mice model
THE SAMP8 MOUSE MODEL

Epigenetics can explain in part the senescent phenotype that characterizes SAMP8
THE SAMP8 MOUSE MODEL

RESULTS

- SAMP8 G9ai
- SAMP8 Control
- SAMR1 Control

**a**

<table>
<thead>
<tr>
<th>kDa</th>
<th>SAMR1</th>
<th>SAMP8</th>
</tr>
</thead>
<tbody>
<tr>
<td>132</td>
<td>G9a</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>GAPDH</td>
<td></td>
</tr>
</tbody>
</table>

**b**

\[
\text{Index (DI)} = 0.8168 \times \text{Duration (s)} + 16.13
\]

**c**

\[
Y = 0.8168 \times X + 16.13
\]

**d**

H3K9me2/H3 total

One-way ANOVA with Tukey post hoc analysis: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

Unpublished results
PHARMACOLOGICAL G9A INHIBITION LEADS TO A REDUCTION IN H3K9ME2 AND AD HALLMARKS, RESTORING DENDRITIC SPINE DENSITY IN SAMP8 MICE

RESULTS

Discrimination Index

\[ t_{(\text{novel})} - t_{(\text{old})} \sim 0 : \text{poor discrimination} \]
\[ t_{(\text{novel})} + t_{(\text{old})} > 0 : \text{increased discrimination} \]
SOME MEMORIES ARE ENCODED IN THE HERITABLE MATERIAL
SOME MEMORIES ARE ENCODED IN THE HERITABLE MATERIAL
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SOME MEMORIES ARE ENCODED IN THE HERITABLE MATERIAL

Where is this memory encoded?
Our brain is complex

100,000,000,000 neurons

7000 synapses per neuron ($10^{15}$)
CAENORHABDITIS ELEGANS

Our brain is complex

100,000,000,000 neurons

7000 synapses per neuron \(10^{15}\)

Expresses the 95% of the genes

A powerful model for studying memory (much simpler brain)

302 neurons - 7000 synapses

Connectome and short life cycle, which is useful for studying the inheritance.

Many molecular pathways like humans
IMPRINTING: A PHASE-SPECIFIC LONG TERM MEMORY

Konrad Lorenz (Nobel Prize, 1973)

Lorenz, 1937; Nevitt et al, 1994; Wilson and Sullivan, 1994
Stable inheritance of an acquired behavior in *Caenorhabditis elegans*

Jean-Jacques Remy

Sensory imprinting produces life-long attachment to environmental features experienced during a critical period of early development. Imprinting of this kind is highly conserved in evolution and is an important form of adaptive behavioral plasticity [1]. The nematode *Caenorhabditis elegans* undergoes such adaptation to new environments through imprinting: attractive odorants, when present during the first larval stage, produce life-long olfactory

would have an improved attraction to olfactory cues if its parent was imprinted. Intriguingly, this appears to be the case: as shown in Figure 1A, a parental imprint was inherited by F1 worms, but not transmitted to F2 worms.

Oliver Hobert and I [2] have previously reported that olfactory imprinting involves at least two classes of neurons, the chemosensory neurons AWC and the interneurons AIV. Imprinting inheritance suggests that neuronal
THE "WEISMANN BARRIER"

Genetic information cannot pass from the soma to the germ line
(The "Weismann Barrier")
EPIGENETIC REPROGRAMMING

August Weismann
1834-1914

E. Heard and R. Martienssen, *Cell* 2014
CAN THE PARENTS’ DIETARY STATE TRANSMIT TRANSGENERATIONALLY?

"The fathers have eaten sour grapes, and the children's teeth are set on edge."

The Dutch Famine (“Hungerwinter”)

(Painter et al. 2008)

Inherited effects of high-fat diet

(Massiera et al. 2010)

INTRODUCTION

Maternal Resveratrol Supplementation Prevents Cognitive Decline in Senescent Mice Offspring

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Resveratrol Supplementation Attenuates Cognitive and Molecular Alterations under Maternal High-Fat Diet Intake: Epigenetic Inheritance over Generations

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CAN NEURONAL MEMORIES TRANSFER BETWEEN GENERATIONS?
Can memories transfer between individuals?

How good are you at remembering something you learned two weeks earlier? What if, during the intervening 14 days, your head was removed? One flatworm isn’t bothered by this scenario. After growing back its entire head and brain, it picks up pretty much where it left off.
EXPERIMENTAL PARADIGM

Design experiment

Does set-25 play an important role in long-term memory after environmental harmful insults?

Unpublished results

Collaboration with Csaba group (Semmelweis University, Budapest)
**EPIGENETIC INHERITANCE**

Olfactory food choice

\[ CI = \frac{(BS - EC)}{(BS + EC)} \]

Intergenerational epigenetic inheritance after 1 imprinting with AM

RESULTS

One-way ANOVA with Tukey post hoc analysis: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

Unpublished results
- GMFB activation mediates the neuroprotection by G9a inhibition.

- Early life toxic stress exposure induced molecular and behavioural changes across generations in *C. elegans* after 1 exposure, through chemotaxis assay and stimulates the expression of the hsp-6 enzyme a toxin-specific cytoprotective.

- Several methyltransferases play an important role in the long-term memory after imprinting intervention
Our work reports a new finding that pharmacological inhibition of G9a might be a promising target for AD therapy, promoting neuroprotection through reduction of its repressive chromatin marks as well as it take part in the epigenetic inheritance memory process.

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