

Functional and pharmacological analysis of the EZH2 protein on olfactory neurogenesis

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Abstract

The main olfactory epithelium (MOE) is one of the few organs in which neurons are constantly renewed, even in adults. Epigenetic factors seem to play an important role in this process and therefore our studies focused on a methyltransferase previously implied in cell proliferation, named EZH2. *In vivo* studies carried out in our lab pointed to a role of the pharmacological inhibition of EZH2 by GSK343 in enhancing the olfactory neuronal population in the MOE. Accordingly, the present study focused on understanding the mechanism involved in this phenomenon, using *in vitro* cell culture of mouse primary olfactory and embryonic stem cells (mESC). We could not obtain stable olfactory stem cells in primary culture, due to slow growth and low yield. For mESCs, treatment with 10 μ M of GSK343 led to lower rates of cell proliferation compared to control. RT-qPCR experiments to test the expression of genes characteristic of proliferating, undifferentiated and differentiated cells are being carried out to further understand the role of EZH2 in olfaction.

Key words:

Neurogenesis, pharmacology, stem cell, olfaction.

Introduction

Understanding the mechanisms by which olfactory neurons proliferate throughout life is of core importance to the development of drugs that can restore a neuronal population in the adult. *In vivo* studies from our lab on Polycomb proteins showed an effect of the EZH2 methyltransferase inhibition on enhancing the number of mature olfactory neurons. Inhibition of this protein led to lower cell proliferation in lymphoma cell lines¹. Thus, a possible mechanism for EZH2 action on the MOE is through the inhibition of stem cell proliferation or differentiation. In this study we performed *in vitro* analyses of EZH2 inhibition on mouse stem cells. To this end, we developed mouse primary olfactory and embryonic stem cell cultures.

Results and Discussion

Firstly, we developed a method for dissection and progenitor cell isolation from the MOE, based on previous publications in our field². Cultures were obtained from the lamina propria or the epithelium itself. A higher number of stem cell-like colonies were observed in the first case, as shown in Image 1, probably due to the location of progenitor cells near the epithelium base. A high number of animals were necessary to yield enough cells for culture. The average time to reach confluence for passage was 3 weeks, which is not similar to tissue growth rate *in vivo* and led us to preclude the use of primary cultures in our study.

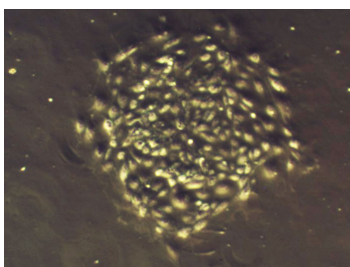


Image 1. Olfactory neuronal stem cells obtained from mouse primary culture, 4 days after dissection (Objective of 10x).

Secondly, in order to test the effects of the EZH2 inhibitor on stem cell development, we carried out experiments on mESC cultures. Different concentrations of EZH2 inhibitor GSK343 were applied to mESCs grown on 12-well tissue culture plates. At 10 μ M, we observed a reduction in cell proliferation rate (Image 2), in agreement with previously reported

effects of the inhibitor on lymphoma cells¹. The enhanced number of mature neurons observed *in vivo* with GSK343 may therefore be linked to EZH2 role on cell differentiation.

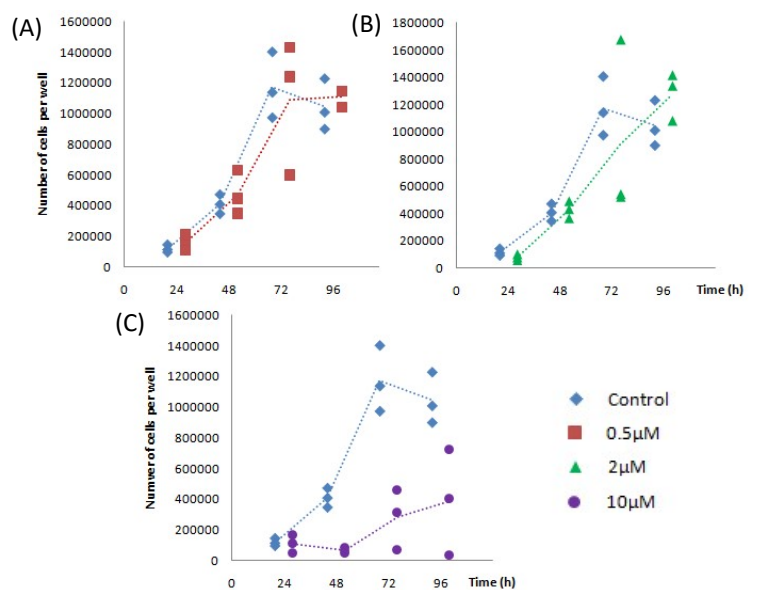


Image 2. mESC cell count using trypan blue method. Cells were cultivated on 12-well plates over 96h and treated with GSK343 in the concentrations of (A)0.5 μ M, (B)2 μ M and (C)10 μ M. Controls received 0.1% DMSO.

Conclusions

EZH2 inhibition by GSK343 led to slower stem cell proliferation. Quantification of gene expression linked to proliferation and differentiation will be performed to elucidate the mechanisms by which EZH2 acts.

Acknowledgement

We thank H. M. Souza for reagents and advice, and the Structural Genomics Consortium for GSK343.

¹ McCabe M.T.; Ott H.M.; Ganji G.; Korenchuk S.; Thompson C.; Van Aller G.S.; Creasy, C. L.EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature*, v.492, 108–112, 2012
² Girard, S. D.; Devéze, A.; Nivet, E.; Gepner, B.; Roman, F. S.; Féron, F.; isolating nasal olfactory stem cells from rodents or humans. *Journal of Visualized Experiments*, 2011 Aug 22 (54).