Investigating the Role of mSlap2 in Gfi1-Mediated Inhibition of Erk1/2 Activation

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Abstract

Gfi1 is known as a nuclear transcriptional repressor that positively regulates B and T cell development and is required for the development of granulocytes. Gfi1 supports granulocyte differentiation at the expense of monocyte differentiation by repressing genes encoding Monocyte-Colony Stimulating Factor (M-CSF) and PU.1, which favors monocyte differentiation. However, the molecular mechanisms by which Gfi1 acts in myeloid development remain incompletely understood. For instance, in contrast to a previous publication reporting that Gfi1 increased the activation of Erk1/2, Dr. Dong’s lab has recently found that Gfi1 inhibits Erk1/2 phosphorylation. The exact pathway leading to Gfi1-mediated inhibition of Erk1/2 activation is yet to be elucidated. As Gfi1 is a nuclear transcription factor, it is possible that Gfi1 may inhibit Erk1/2 activation by regulating the expression of a cytoplasmic regulator of Erk1/2 activation. In preliminary data obtained from Dr. Dong’s lab, Gfi1 was shown to upregulate the expression of mSlap2. Interestingly, mSlap2 is a cytoplasmic protein that has been shown to inhibit the activation of Erk1/2. We hypothesize that mSlap2 may play a key role in Gfi1-mediated inhibition of Erk1/2 activation.

Figure 1: Effect of Gfi1 deficiency on Erk1/2 activation in bone marrow cells.

(A) RT-PCR was performed to amplify mSlap2 cDNA from BaF3 and 32D cells. PCR products were run on 1.0% agarose gel. (B) Depicted above is the map of pGEM-T Easy vector used for cloning of mSlap2 cDNA. (C) Plasmid DNA was isolated from individual bacterial clones and digested with EcoRI to confirm the insertion of mSlap2 cDNA into pGEM-T Easy vector.

Figure 2: A Role of Gfi1 in the Regulation of mSlap2 Expression

(A-B) Myc epitope-tagged Gfi1 overexpression in BaF3 cells results in increased expression of mSlap2. (C) BaF3/Gfi1 cells transiently transfected with mSlap2, Myc epitope tag

Figure 3: Deletion of mSlap2 Stop Codon

(A) The stop codon in mSlap2 cDNA was removed by mutagenesis, which generated a restriction site for Apal. (B) Plasmid DNA isolated from individual bacterial clones and digested with Apal to confirm the insertion of mSlap2 cDNA into pGEM-T Easy vector.

Figure 4: Insertion of mSlap2 cDNA into pGEM-T Easy Cloning Vector

(A) RT-PCR was performed to amplify mSlap2 cDNA from BaF3 and 32D cells. PCR products were run on 1.0% agarose gel. (B) Depicted above is the map of pGEM-T Easy vector used for cloning of mSlap2 cDNA. (C) Plasmid DNA was isolated from individual bacterial clones and digested with EcoRI to confirm the insertion of mSlap2 cDNA into pGEM-T Easy vector.

Figure 5: Deletion of mSlap2 Stop Codon

(A) The stop codon in mSlap2 cDNA was removed by mutagenesis, which generated a restriction site for Apal. (B) Plasmid DNA isolated from individual bacterial clones and digested with Apal to confirm the presence of the mutation.

Figure 6: Insertion of mSlap2 into Mammalian Expression Vector pcDNA 3.1/myc-His(+) A

(B) Apal Digestion

C.

D.

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Performed RT-PCR

Synthesized cDNA

Inserted mSlap2 into pGEM-T Easy Cloning Vector

Performed mutagenesis on mSlap2

Inserted mutated mSlap2 into pcDNA3.1/myc-6xHis(A) mammalian expression vector

Figure 1: Effect of Gfi1 deficiency on Erk1/2 activation in bone marrow cells.

Lineage marker negative (Lin) BM cells were isolated from Gfi1+/+ and Gfi1−/− mice and treated with G-CSF, M-CSF for the indicated times. Erk1/2 phosphorylation was examined by Western blot analysis. This data was graciously provided by Dr. Fan Dong.

Parts of the project accomplished Summer 2017

Parts of the project to be completed in Fall 2017-Spring 2018