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THE SILENCING OF IBA-1 CHANGES THE PHYSIOLOGY OF MICROGLIA

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INTRODUCTION

Iba-1 (Ionized calcium binding adaptor molecule 1), also known as Aif-1, is a 17 kDa microglia-specific protein which is encoded by AIF1 gene. Iba-1 is a marker associated with activated microglia and is involved in key functions such as phagocytosis and migration, being an important structure of the cytoskeleton. Previous studies showed that Iba-1 forms complexes with L-fimbrin in membrane ruffles which are involved in modulating actin reorganization to facilitate cellular motility and phagocytosis by microglia (Ohsawa et al., 2004). The goal of this study was to evaluate how the silencing of Iba-1 in BV2 cells could change the characteristic functions of microglia.

MATERIALS AND METHODS

BV2 microglia cell line transfected with 40 nM scramble or Iba-1 siRNA using Lipofectamine RNAiMAX for 24h, was left to recover and increase Iba-1 silencing for another 48h. Through PCR the mRNA level was analyzed and western-blot revealed the reduction at the protein level. The migrations and phagocytosis were evaluated also at 72h after transfection. Using Benzoyl-ATP, the calcium influx through P2X7r was recorded to test the effect of Iba-1 silencing on the ionic channel activity.

RESULTS

The Iba-1 silencing was confirmed both through PCR and western-blot 72h after transfection, using different concentrations of small interfering RNA. The silencing significantly reduced the transmigration, chemotaxis, chemokinesis and invasion of BV2 cells, using Boyden chambers with 8 μm pores. On the other hand, the phagocytosis of the fluorescent beads was not affected after the silencing. In addition, the analysis of the BV2 subpopulation transfected with a reporter Alexa594-siRNA in 1:10 ratio compared to target siRNA, did not show the same effect on migration and phagocytosis, most likely due to a reduced population size. The ratiometric calcium imaging with Fura2 using 300 uM Benzoyl-ATP, a selective agonist of P2X7, revealed a decrease of the calcium signal after Iba-1 silencing compared with the scramble condition.

CONCLUSIONS

The Iba-1 silencing affects the functioning of BV2 cells, reducing the calcium signal through P2X7r and the migration rate, suggesting a new mechanism through which the physiology of microglia could be altered for therapeutic purposes.