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Role of MAP kinase ERK5 in Neuroblastoma chemoresistance and chemosentivity

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Introduction

Neuroblastoma (NBL) is the most common pediatric solid tumors, and it accounts for 15% of all pediatric cancer deaths. Despite approaches made to treat this disease, the standard therapy for high-risk NBL has not changed over the last years and many of high-risk patients do not response to conventional therapies or relapse after treatment. This poor prognosis is associated with metastasis and multi-drug resistance. Therefore, there is clear need of new and better therapies to tackle neuroblastoma (NBL).

The extracellular related kinase 5 (ERK5/MAPK7/Bmk1) is the most recently described MAP kinase, and is activated in response to a wide range of growth factors and cellular stresses. ERK5 controls many cellular processes such as growth, proliferation, and survival, among others. ERK5 has a particular structure that differentiates it from other mammalian MAPKs. It has an N-terminal kinase domain closely related to ERK2, but it also has a unique C-terminal tail that contains a nuclear localization signal (NLS) and a transcriptional activation domain. ERK5 has been proposed as a novel therapeutic target in cancer, since either ERK5 inhibitors or its silencing induce cell death in cancer cells;however, the efficacy of these treatments in pediatric solid tumors has not been explored.

Objectives

Since ERK5 plays an essential role in neuronal development and plasticity and it has been proposed as a novel therapeutic target in cancer, we aimed to explore the therapeutic impact of inhibition of ERK5 kinase activity or ERK5 expression on pediatric neuronal tumors such as NBL.

Methods

Experiments were performed using a panel of clinically-representative human NBL cell lines. Silencing of ERK5 experiments were performed using two different lentiviral shRNAs against human ERK5. Pharmacological inhibition of ERK5 kinase activity was achieved using the specific inhibitor XMD8-92. Cell viability and apoptosis were measured with the MTT method, Hoechst staining and annexin V/PI staining. Cytosolic and nuclear localization of ERK5 was visualized by subcellular fractionation as wells by as by immunofluorescence experiments. ERK5 expression was analyzed by immunoblotting. Expression of constitutive nuclear form of ERK5 was achieved by stable lentiviral transduction in different NB cell lines. To do so, a lentiviral vector was generated, ERK5-5E, containing the five autophosphorylatable residues of the C-term tail S567, S720, S731, T733 and S803 were mutated to glutamic acid, which results in constitutive nuclear form of ERK5. Changes on human transcriptome in response to ERK5 silencing was performed in two chemoresistant NBL cell lines using the Clariom S Assay (Applied Biosystems).

Results

Here we show that chemo-sentitive NBL cell lines show cytosolic ERK5 and sensitivity to ERK5 inhibitors. Moreover, ERK5 inhibition synergizes with cisplatin chemotherapy to promote NBL cytotoxicity. Mechanistically, we show that ERK5 regulates transcription of p53 in chemo-

sentitive NBL cells, and therefore ERK5 inhibition results in p53-mediated activation of apoptosis. On the other hand, chemo-resistant NBL cells show constitutive nuclear ERK5 and resistance to ERK5 inhibitors. These cells, however, are sensitive to ERK5 silencing, which activates apoptotic cell death. We have performed DNA microarray analysis to identify genes involved in activation of apoptosis by ERK5 silencing in chemo-resistant NBL cells. Interestingly, expression of a constitutive nuclear form of nuclear ERK5 in chemo-sentitive NBL results in cells that are resistant to chemotherapy (cisplatin), indicating that nuclear ERK5 might play an important role in acquisition of resistance in NBL cells.

Conclusions

ERK5 plays a dual role on proliferation and viability of neuroblastoma, dependently or independently of its kinase activity. NBL poor prognosis correlates with nuclear ERK5, where it plays a role in acquisition of chemoresistance. We propose that p53+ NBL tumors might benefit from combining treatment of standard chemotherapy together with ERK5 kinase inhibitors. On the other hand, p53- chemoresistant NBL tumors are resistant to ERK5 inhibitors but sensitive to ERK5 silencing. Therefore, alternative approaches must be developed to target ERK5 protein in neuroblastoma.

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