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Anti-tumor activity of indocarbazole analogs in sarcoma

Óscar Rafael Estupiñán Sánchez[2] , Lucía Fernández Nevado[1] , Verónica Rey Vázquez [1] , Sofía Tirados Menéndez[2] , Laura Santos[1] , Aida Rodríguez[1] , Francisco Morís[3] , René Rodríguez[1]

1) Hospital Universitario Central de Asturias - Instituto de Investigación Sanitaria del Principado de Asturias, Oviedo, Spain - Instituto Universitario de Oncología del Principado de Asturias, Oviedo, Spain. 2) Hospital Universitario Central de Asturias - Instituto de Investigación Sanitaria del Principado de Asturias, Oviedo, Spain - Instituto Universitario de Oncología del Principado de Asturias, Oviedo, Spain - CIBER de Cáncer (CIBERONC), Madrid, Spain 3) EntreChem SL, Oviedo, Spain

Introduction

Sarcomas comprise a heterogeneous group of aggressive mesenchymal malignancies that often show a limited clinical response to cytotoxic drugs which remain as the most utilized agents for first-line treatment of soft tissue sarcomas. Survival rates for patients presenting with metastatic and recurrent disease have historically remained essentially unchanged with a survival rate below 20%, highlighting the need for new, more efficient, therapeutic approaches.

Multi-kinase inhibitors like the indocarbazole analogs Midostausin and EC-70124 have provided an efficient way to target several pro-tumorigenic pathways using a single agent and have shown anti-tumor activity in wide range of tumors. Midostaurin have been recently approved by the FDA for the treatment of acute myeloid leukemia and EC-70124 is ongoing advanced preclinical studies, having proved efficacy in colorectal tumors, breast cancer, prostate cancer and glioblastoma pre-clinical models. Sarcomas, often show aberrant activation of pro-tumoral kinase activities, however, the effect of multikinase inhibitors in sarcoma have not been tested yet.

Objetives

In this work we aimed to study the effect of EC-70124 and Midostaurin in cell-of-origin sarcoma models (originated from transformed human mesenchymal stem cells) and sarcoma patient-derived primary cell lines. We correlated the anti-tumor activity of these drugs with their ability to inhibit a panel of kinases in order to gain insight of their mechanisms of action.

Methods

The cytotoxic effect of indocarbazole analogs and other pathway-specific inhibitors was characterized by analyzing their ability to reduce cell survival (WST-1 cell proliferation assays), to induce apoptosis (PARP cleavage), to induce cell cycle arrest (FACS analysis) and/or to induce DNA damage (immunofluorescence analysis of ?-H2AX levels).

The effect on activated signalling proteins was studied using phospho-antibody arrays and Western-blotting analysis. The possible existence of synergism between indocarbazole analogs and other pathway-specific inhibitors, which may provide information regarding the mechanism of action of EC-70124 and Midostaurina in sarcomas, was analyzed using the CompuSyn software to calculate the Combination Index according to the Chou-Talalay method.

Results

In striking contrast, cell-of-origin sarcoma models were sensitive to sub-micromolar concentrations of EC-70124 (IC_{50} between 0.17 and 0.34 ?M) while were resistant to Midostaurin treatment. Cell cycle analysis showed that EC-70124 treatment induced a slow transition through the S-Phase followed by G2 arrest, DNA damage induction and apoptosis. On the other hand, Midostaurin treatment is characterized by a G2 arrest and endo-replication leading to the generation of polyploid cells, without presenting S-phase delay nor a significant apoptotic induction.

Evaluation of the phosphorylation/activation status of several receptor tyrosine kinases (RTKs) and downstream signalling mediators evidenced that EC-70124 was much more efficient than Midostaurin inhibiting protein kinases in sarcomas. Specifically, components of the PI3K/mTOR pathway, such as phospho-AKT and phospho-S6, were among the most activated kinases in sarcoma models and were the targets most efficiently inhibited by EC-70124. The relevance of the inhibition of mTOR pathway on the mechanism of action of EC-70124 was evaluated by exposing the sarcoma models to the specific mTOR inhibitor Torin 1. This inhibitor efficiently reduced phospho-S6 and phospho-AKT levels and, at the highest doses, induced approximately a 60% of the cell toxicity produced by EC-70124. Torin 1 showed a strong synergist effect when combined with Midostaurin while only a weak synergistic effect when combined with EC-70124, thus suggesting that a strong inhibition of mTOR signalling may be a relevant mechanism of action for EC-70124 in sarcomas, while the failure of Midostaurin to inhibit this pathway might explain its lack of cytotoxicity.

To confirm the potential of EC-70124 as anti-tumor agent for sarcomas we tested their efficacy in a panel of patient-derived primary cell lines. We found that most analyzed cell lines expressed high levels of phospho-S6, phospho-AKT, phospho-STAT1 and phospho-STAT3, and that EC-70124 efficiently reduced the levels of these phospho-proteins. According to this ability to inhibit PI3K/mTOR and JAK/STAT signalling, EC-70124 was able to induce dose-dependent cell toxicity in all cell lines.

Conclusions

EC-70124, opposite to other indocarbazole analogs like Midostaurin, is able to efficiently inhibit kinase activity and to induce a strong cytotoxic response in sarcomas through a mechanism involving mTOR pathway inhibition.