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Application of the SORE6 reporter system on the screening of anti-cancer stem cell drugs in sarcoma models

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Introduction

Metastasis, together with tumour recurrence and drug resistance, are the main causes of mortality in patients with cancer. Despite major advances in radiotherapy and chemotherapy; conventional treatments are unable to prevent tumour progression. Even though these therapies can effectively target proliferating cells, thus contributing to tumour reduction, they are unable to successfully eradicate cancer stem cells (CSCs). Increasing evidences highlight the pivotal role that CSCs play in tumour progression. Due to their ability to self-renew, resist different therapeutic strategies or disseminate and initiate tumor growth at distant sites, targeting CSCs represents a great challenge in order to improve clinical response in cancer patients. Their elimination could not only contribute to impair tumour growth, but also drug resistance and metastasis. Unfortunately, there is not a suitable methodology to routinely isolate and detect CSCs.

Higher expression levels of genes related to pluripotency and self-renewal, like OCT3/4 or SOX2, are a typical feature of CSCs. Previous work from our group demonstrated that SOX2 expression increased in CSCs populations during tumour progression (Martinez-Cruzado L et al. Sci Rep. 2016. doi: 10.1038/srep27878), hence highlighting its potential applicability as CSC marker in sarcomas. The laboratory of Dr L. M. Wakefield designed a lentiviral-based reporter system in which a composite SOX2/OCT4 response element (SORE6), coupled to a minimal cytomegalovirus (CMV) promoter, controls the expression of GFP or mCherry fluorescent reporter genes (Tang B et al. Stem cell reports. 2015. doi: 10.1016/j.stemcr.2014.11.002). Cancer cells expressing this system can be used to detect and isolate viable cells expressing transcriptionally active SOX2 and/or OCT4 by flow cytometry or fluorescence microscopy.

Objective

This work aimed to study the applicability of SORE6 reporter system as a novel tool to evaluate the effectiveness of novel and traditional anti-tumour treatments in the eradication of CSCs in sarcoma both *in vitro* and *in vivo*.

Methodology

We used a xenograft-derived cell line (T5H-O) generated by a cell-origin-model of undifferentiated pleomorphic sarcoma developed from transformed Bone Marrow Mesenchymal Stromal/Stem Cells (BM-MSCs). This model has proven very useful to explore the evolution of CSCs subpopulations and to search for CSC-specific markers and therapies. In addition, we used a primary patient-derived chondrosarcoma cell line (CDS17) as well a cell line (T-CDS17) derived from a xenograft generated by CDS17 cells. T5H-O, CDS17 and T-CDS17 cells were transfected with the lentiviral-based reporter system SORE6. Subsequently, we evaluated the the ability of the drugs to target SORE6 positive cells both in cell cultures or in mice bearing T-5H-O-SORE6 tumors using time-lapse microscopy and/or flow cytometry.

Results

First we verified that the SORE6 construction was able to identify a subpopulation with stem cell like properties in our sarcoma model (T5H-O). After sorting, SORE6 positive (+) cells showed enhanced growth in tumoursphere cultures and also increased invasion ability than the SORE6 negative (-) population. Importantly, *in vivo* analysis demonstrated that SORE6+ cells were significantly more tumorigenic than the SORE6- population, thus indicating that SORE6 activity marks a subpopulation with increased tumour-promoting ability in sarcomas. Then we treated T5H-O-SORE6 cells with trabectedin, doxorubicin and paclitaxel, currently in clinical use for the treatment of malignant tumours, and with EC-8042, a mithramycin (MTM) analogue which have previously demonstrated its potential to target CSC subpopulations in sarcoma (Tornin J et al. Oncotarget. 2016. doi: 10.18632/oncotarget.8817). We found that EC-8042 was the drug that more efficiently reduced the SORE6+ subopulation. In addition, we found that EC-8042-treatment sharply reduced the percentage of

SORE6+ cells even before the appearance of apoptosis, thus suggesting that this drug is able to suppress the transcriptional activity responsible of SORE6 activity. Similarly, EC-8042 was the only drug able to decrease the percentage and activity of SORE6+ cells in vivo.

Conclusions

Our results indicate that the SORE6 reporter is an effective tool to identify stem-cell subpopulations in sarcoma. This reporter system also constitutes an excellent approach for testing the effectiveness of CSC-specific treatments.

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