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Short communication

Medium throughput biochemical compound screening identifies novel agents for pharmacotherapy of neurofibromatosis type 1

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ABSTRACT

The variable manifestation of phenotypes that occur in patients with neurofibromatosis type 1 (NF1) includes benign and malignant neurocutaneous tumors for which no adequate treatment exists. Cell-based screening of known bioactive compounds library identified the protein phosphatase 2A (PP2A) inhibitor Cantharidin and the L-type calcium channel blocker Nifedipine as potential candidates for NF1 pharmacotherapy. Validation of screening results using human NF1-associated malignant peripheral nerve sheath tumor (MPNST) cells showed that Cantharidin effectively impeded MPNST cell growth, while Nifedipine treatment significantly decreased local tumor growth in an MPNST xenograft animal model. These data suggest that inhibitors of PP2A, as well as calcium channel blockers, might be used in broader MPNST preclinical studies as single agents or in combinatorial therapeutic strategies.

1. Introduction

Neurofibromatosis type 1 (NF1), also known as *von Recklinghausen's disease,* is a frequent human cancer predisposition syndrome affecting many organ systems, peripheral nervous system in particular [1–3]. Typical NF1 symptoms include neuroectodermal tumors and lesions of other tissue origins, pigmentation patterns, as well as skeletal abnormalities and cognitive deficits [3]. Most NF1 patients develop dermal and plexiform neurofibromas - benign lesions associated with peripheral nerves. Dermal neurofibromas are tumors in the skin, while plexiform neurofibromas (PNF) develop along the nerve plexus. PNFs can reach large sizes and compress nearby nerves, causing pain and a range of dysfunctions; moreover, PNF can transform into malignant peripheral nerve sheath tumors (MPNSTs) [4]. These cancers exhibit highly aggressive metastatic growth and serve as major source of morbidity for NF1 patients [4,5]. To date there is no established pharmaceutical treatment for benign and malignant NF1-associated tumors, thus, the search for novel therapeutic targets for this disease is of intense scientific and clinical interest [6].

Neurofibromatosis type 1 is a monogenic dominantly inherited autosomal disorder. It occurs due to inactivating mutations in the *NF1* tumor suppressor gene that encodes the Ras-regulatory protein neurofibromin [6,7]. Neurofibromin is broadly expressed and plays important roles in regulating multiple cellular processes, as evidenced by the variety of symptoms arising from its loss [3,6]. However, specific functions of neurofibromin remain unclear. Although many neurofibromin interactions with other proteins have been reported in addition to controlling Ras [3], the biological meaning of these additional interactions is largely undefined.

Genetic and biochemical screenings can be useful tools for defining new protein functions as well as therapeutic targets. Here we perform cell-based phenotypic screening assay to identify novel compounds that inhibit proliferation and survival of cells lacking neurofibromin. We show that small molecules of two distinct classes – the protein phosphatese 2 (PP2A) inhibitor Cantharidin and the calcium antagonist Nifedipine - exhibit selective toxicity towards NF1-deficient mouse embryonic fibroblasts (MEFs). We demonstrate that Cantharidin effectively inhibits growth of human NF1-associated MPNST cells, suggesting that PP2A might represent an attractive new target for MPNST pharmacotherapy. Finally, we show that Nifedipine inhibits cell growth as well as xenograft

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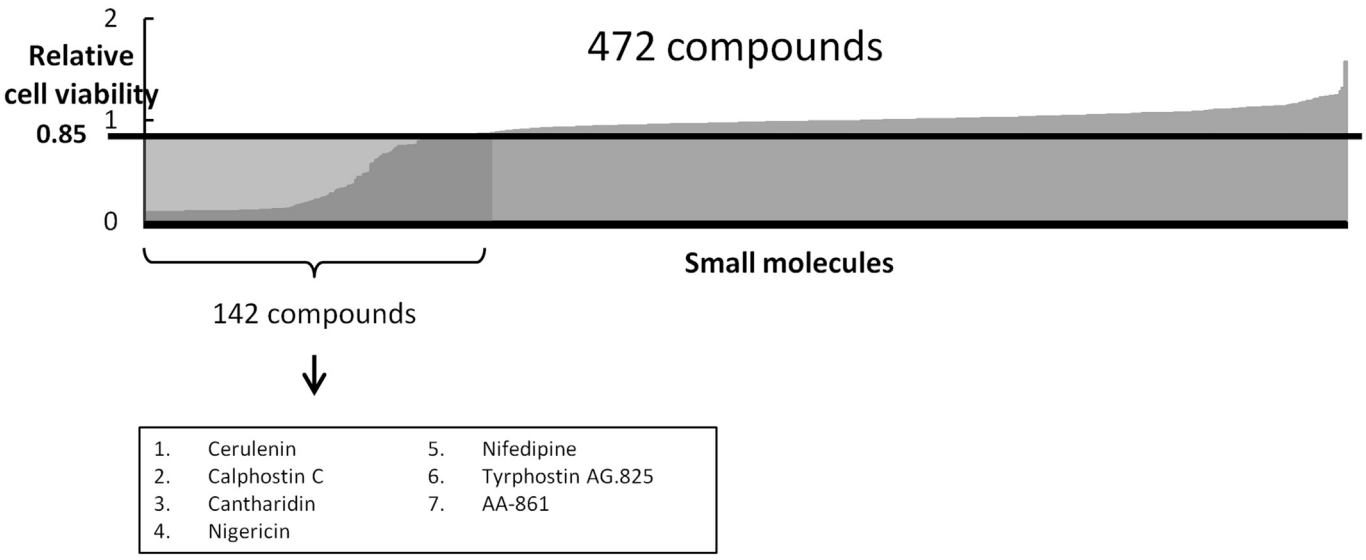
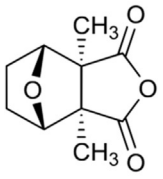
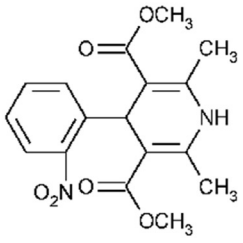
A

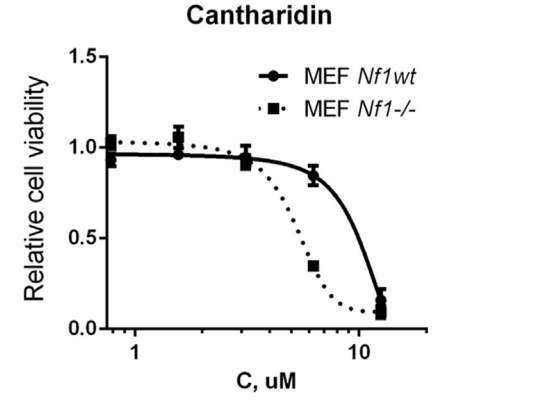


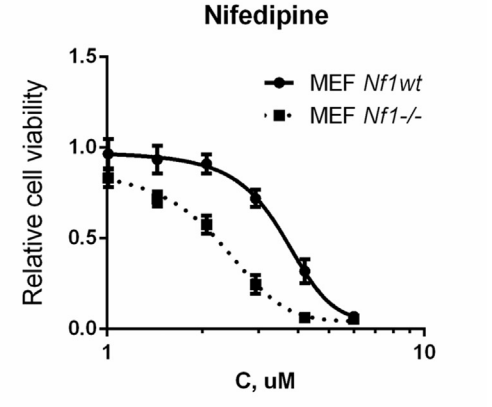
1.	Cerulenin	5.	Nifedipine
2.	Calphostin C	6.	Tyrphostin AG.825
3.	Cantharidin	7.	AA-861
4.	Nigericin		

B









IC50(MEF *Nf1wt*) = 8.52 ± 0.58 uM
IC50(MEF *Nf1-/-*) = 5.04 ± 0.21 uM | ***

IC50(MEF *Nf1wt*) = 3.69 ± 0.40 uM
IC50(MEF *Nf1-/-*) = 2.39 ± 0.23 uM | ***

C

	88-3	ST8814	90-8	S462TY	sNF96.2	sNF94.3	sNF02.2
Cantharidin IC50, uM	2.83 ±0.32	3.38 ± 0.27	3.04 ± 0.17	4.25 ± 0.26	1.57 ± 0.13	0.92 ± 0.14	2.88 ± 0.22
Nifedipine IC50, uM	> 20	> 20	> 20	0.32 ± 0.03	> 20	> 20	> 20