Letter to the Editor

Cancer Research

Targeting Taspase1 for Cancer Therapy—Response

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We appreciate the interests of Stauber and colleagues in our study, identifying a small-molecule Taspase1 inhibitor (TASPIN) NSC48300 that disrupts cancer cell growth (1). First, regarding the expression level of Taspase1 in 293T cells, Taspase1 was first purified from 293T cells on the basis of the presence of potent proteolytic activity (2). Therefore, the claim that 293T has no measurable expression of Taspase1 is incorrect. The activity of Taspase1 in 293T cells is quite evident as the dual fluorescent proteolytic reporter (GFP 2XNES-MLL(2500-2900)-3XNLS dsRED2) is efficiently processed in 293T cells, resulting in red fluorescence in the nucleus and green fluorescence in the cytosol (Fig. 3; ref. 1). This assay was established in 2004 and used to screen for bioactive TASPINs (1).

Second, regarding the Taspase1 cleavage consensus motif (IXQL(V)D/G) and the importance of hydrophobic residue at the P5 position, to fully understand the biologic function of Taspase1, we have generated Taspase1 knockout mice (3) and tried various *in silico* methodologies, hoping to identify additional Taspase1 substrates. Disappointingly, all candidate substrates identified through *in silico* approaches failed to pass our established *in vitro* and *in vivo* Taspase1 cleavage assays. Hence, we have discarded such an approach for many years. We were quite intrigued by the success using an *in silico* approach by Bier and colleagues in identifying many Taspase1 substrates, among which USF2 was best characterized (4). As MLL1, MLL2, TFIIA, and ALF are *bona fide* Taspase1 substrates identified so far (3, 5), we wished to expand the Taspase1 substrate repertoire and thus examined whether USF2 is a Taspase1 substrate. As pointed out by Stauber and colleagues, USF2 does not carry a hydrophobic residue at P5. We first conducted an *in vitro* cleavage assay using *in vitro* transcribed and translated full-length USF2. Unlike MLL and TFIIA that are efficiently processed by purified recombinant Taspase1 *in vitro*, USF2 was not cleaved. We then examined the cleavage of USF2 *in vivo* using 2 different assays using *Taspase1*$^{+/+}$ and *Taspase1*$^{-/-}$ mouse embryonic fibroblasts. In consistence with our *in vitro* data, we did not detect processing of endogenous and exogenously expressed USF2 in *Taspase1*$^{+/+}$ cells.

Third, as NSC48300 is an arsenic acid containing compound, it is foreseeable that it has additional targets other than Taspase1, which were discussed in our article (1). Accordingly, collaborative efforts are undertaken by the Chemical Biology Consortium through the National Cancer Institute to identify additional TASPINs for potential clinical application.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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