

Protein Conformational Array Technology Identifies SHP2-Degradation Molecules

Array Bridge is testing promising drug candidates that can effectively bind and even degrade target proteins, but not via their active sites

By Xing Wang and Griffen Brock

Cancer is one of the leading causes of death worldwide, affecting over 20 million individuals and killing 9.7 million in 2022 alone. Each of ~200 cancer types (and multiple subtypes) presents an overwhelming number of possible drug targets. Orthosteric inhibitors, or active-site inhibitors, have become more useful in the last few decades, often producing positive outcomes when directed to the correct target.

Although inhibiting a protein target by active-site binding can be effective, it is also challenging or even impossible when targeting certain proteins such as enzymes with low steric specificity or functional proteins without an active site.

These challenges, along with poor bioavailability, are leading researchers to take different approaches such as allosteric inhibition, i.e., molecules that bind to protein targets at sites away from the active site, yet still perform functional inhibition.

Significant interest

One cancer target of significant interest, a non-receptor protein tyrosine phosphatase (PTP) known as Src Homology 2 domain-containing protein tyrosine phosphatase (SHP2), is one of these challenging yet important targets. Coded by the oncogene PTPN11, SHP2 plays an important role in multiple cell-signaling pathways, acting as a regulator for cytokines and growth factors. With influence on JAK/STAT, P13K/AKT, and RAS/RAF/ERK pathways, the effects of SHP2 variants can be traced to an extensive list of diseases like breast cancer, lung cancer, lymphomas, glioblastomas, and colon cancer. This long list of affected pathways and cancers has earned the oncoprotein a “pleiotropic” label; a molecule/gene that plays multiple regulatory roles within multiple pathways and is often responsible for multiple phenotypic outcomes within cells.

The complicated multi-domain structure of SHP2 supports its wide functional range, with the N-SH2, C-SH2, and PTP (protein-tyrosine-phosphatase) domains acting coordinately for activation. SHP2 will often auto-inhibit when phosphorylated, using the N-SH2 domain as a “latch” onto the PTP domain. This is how SHP2 functions in normal cells, performing required actions but self-inhibiting if necessary. In some cancerous cells, SHP2 is unable to auto-inhibit and is stuck in the “active” mode, causing extreme proliferation, decreasing apoptosis, and leading to tumor growth and metastasis.

This malfunction, resulting from mutations in the PTPN11 gene, inhibits the SHP2 domains from interacting with each other correctly and leads to a gain of function. SHP2 is not uniformly expressed in cells; for instance, hematopoietic cells express SHP2 at much higher levels than other cells, but its function is not affected by this. Whether over- or underexpressed, SHP2 expression may be an important biomarker for certain diseases.

Identification of SHP2-degradation molecules with PCA

SHP2 inhibition could be an ideal way to treat certain cancers, and degradation of the protein is one of the promising approaches. Allosteric inhibition is currently preferable for SHP2 because of challenges presented by active-site inhibition; catalytic site inhibitors are known to have undesired selectivity against SHP1 and other PTPs (PTP1B, DEP1, etc.), as well as ionizable functional groups effecting successful cell permeability.

PCA (Protein Conformational Array) technology can quantify conformational change by measuring protein surface epitope disruption. Using a panel of antibodies raised against the 27 overlapping peptides covering the whole amino acid sequence of SHP2, a quantifiable molecular “fingerprint” can be created as a histo-

gram. Subsequent PCA screening identified 15 compounds from the NCI Diversity Compound Library that elicited significant conformational changes in SHP2 proteins. These 15 compounds were then tested against six different cancer cell lines *in vivo*. To quantify the impact of compound treatment on SHP2, gel electrophoresis and western blotting were performed for each of the cell line/drug compound com-

binations (Figures 1 and 2).

Figure 1 illustrates that SHP2 expression level can influence the effective inhibition of the candidate drug/compound being tested. In samples where SHP2 expression is up-regulated (Cell Lines 1+3), there was an increase in candidate drug effectiveness with SHP2 degradation. However, in samples where SHP2 was expressed at a lower level (Cell Lines

2+4), there was an up-regulation in SHP2 expression resulting from drug treatment. These results suggest that measuring the expression level of SHP2 could be vital when choosing a drug for the target.

While lower expressions of SHP2 in cells are up-regulated after drug treatment, cells with highly expressed SHP2 demonstrate significant inhibition of SHP2. Inhibition of highly expressed SHP2 cell lines is shown in Figure 2. Column 1) shows naturally expressed SHP2 while columns 2–16 show each compound's effect on the cell line expression. A handful of compounds induced SHP2 degradation in all 3 cell lines (Figure 2), a first among allosteric inhibitors. The data shown in Figure 1 and Figure 2 suggest that treatment outcomes will vary among cells with unique SHP2 expression levels.

Conclusion

Of the hundreds of catalogued potential oncoproteins in human cells, SHP2 stands out as an important driver of tumor growth for many cancers. As a pleiotropic molecule affecting numerous cellular pathways, SHP2 inhibition can play an important role in current combination therapies against certain cancers and other diseases. Although SHP2 allosteric inhibitors are being developed and tested in many cells and animal models, this report is the first to demonstrate SHP2 degradation by allosteric regulators. With its close involvement in multiple cancer types, effective degradation of SHP2 proteins could be a promising approach for cancer treatment. **GEN**

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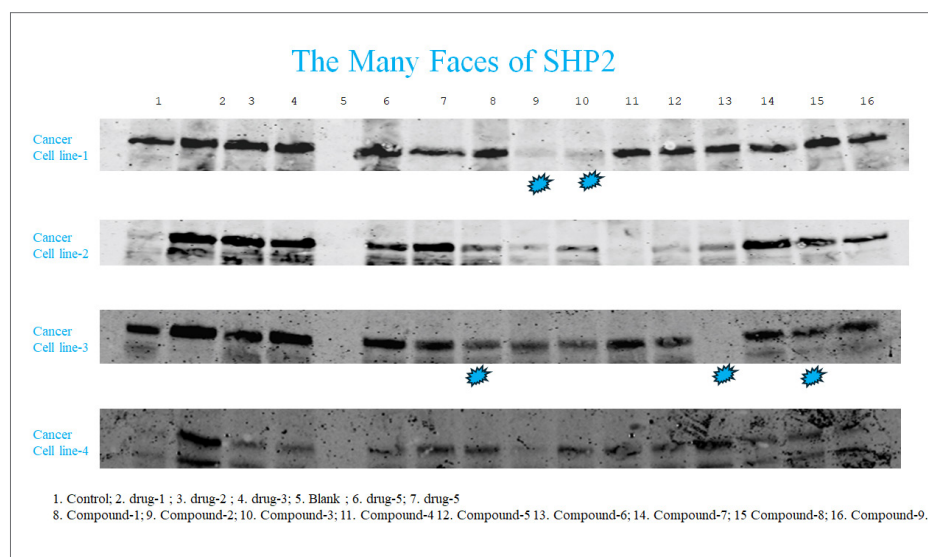


Figure 1. Western blot of four cell lines tested against 15 compounds, showing how normal SHP2 expression levels could be a marker for drug effectiveness. Blue splotches indicate reduced expression in the specific compound/cell line combination.

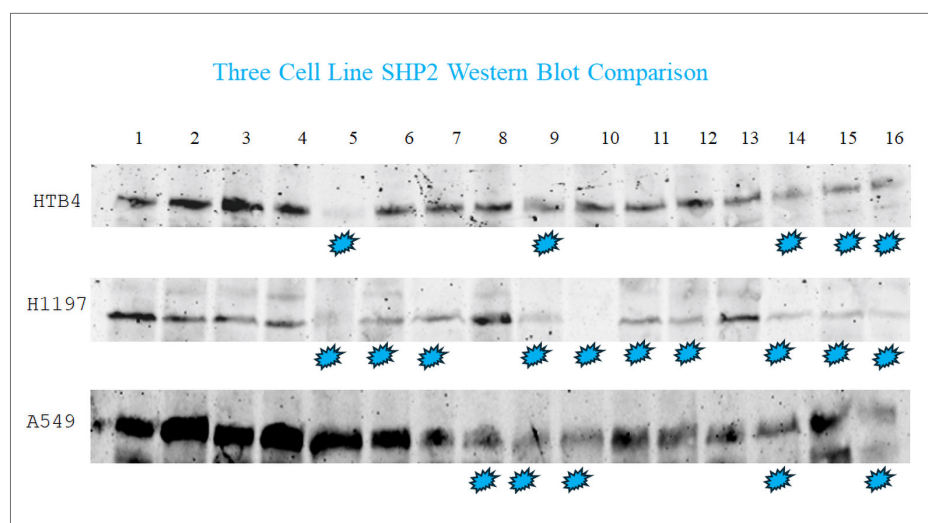


Figure 2. Western Blot of three cell lines tested by 15 compounds; blue splotches indicate an inhibition or degradation of the marked cell line/candidate drug combination. Compound-induced degradation can be seen throughout all three cell lines above.