Selective Targeting of Sirtuin Isoforms for Cancer Therapeutics

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Sirtuins are a family of proteins involved in multiple aspects of cellular function. Humans encode seven sirtuin homologs, which play important roles in aging, diabetes, neurodegenerative disorders, and cancer. However, "the biggest challenge in the field has been to determine whether modulation of sirtuin activity can be used for therapeutic benefit," according to Dr. Julian Simon in the Clinical Research and Human Biology Divisions. A new study from the laboratories of Dr. Simon and Dr. Antonio Bedalov (Clinical Research Division), published in the *Journal of Medicinal Chemistry*, describes the development of selective inhibitors to three of the human sirtuin isoforms and shows that selective inhibition of SIRT2 demonstrated anticancer activity.

Sirtuins function as protein deacetylases, removing acetyl groups from histones and other proteins to regulate aging, metabolism, and development. Both human isoforms SIRT1 and SIRT2 deacetylate and inhibit the function of tumor suppressor proteins, including p53. Inhibiting tumor suppressor proteins drives cancer formation by promoting the survival of cells that have DNA damage or have accumulated oncogenic mutations. Previously, the Simon lab identified a relatively non-selective sirtuin inhibitor, cambinol, which inhibits both SIRT1 and SIRT2 isoforms by competing for substrate binding (Heltweg *et al*., 2006). Cambinol blocked lymphoma cell growth *in vitro* and *in vivo*, and blocked the removal of acetyl groups from the SIRT1 target p53 and the SIRT2 target α-tubulin.

In the current work, the Simon laboratory aimed to develop more selective sirtuin inhibitors to elucidate which specific sirtuin isoform is a valid anti-lymphoma drug target. The researchers used medicinal chemistry to determine what effect structural changes have on the desired biological activity of cambinol, the so-called Structure Activity Relationship or SAR. To guide the structural changes in the chemical compound, the researchers first mapped where the inhibitor interacted with the SIRT1 protein using saturation transfer difference (STD) NMR. Newly generated compounds were first tested using purified recombinant SIRT1, SIRT2, and SIRT3 enzymes. The researchers developed one compound with >7.8-fold selectivity for SIRT1 and another with >15.4-fold selectivity of SIRT2. A third compound was potent against SIRT3, and also inhibited SIRT1 or SIRT2 at higher concentrations.

These three inhibitors were then tested in cell-based assays with a Burkitt's lymphoma cell line, looking at cell viability after drug treatment and the induction of apoptosis. The researchers found that inhibition of SIRT2, but not SIRT1 or SIRT3, correlates with anti-lymphoma activity (r=0.56, p=0.0014). The three compounds were tested for cellular cytotoxicity against a larger panel of lymphoma cell lines, as well as breast, colon, and lung cancer cell lines and compared to normal, untransformed B cell lines. The SIRT2-selective inhibitor had an inhibitory concentration killing 50% of cells (IC50) of 3 to 7 micromolar in the cancer cell lines compared to 22 to 28 micomolar in the non-transformed B-cell lines.

Overall, this study demonstrated sirtuin inhibitors are valid anticancer agents. According to Dr. Simon, "we now have a better idea of how to keep improving this compound series." Based on results from the STD NMR experiments, the researchers identified positions in the chemical compound as potential sites for improving selectivity and potency of inhibitors for the various sirtuin isoforms.

Mahajan, S.S., Scian, M., Sripathy, S., Posakony, J., Lao, U., Loe, T.K., Leko, V., Thalhofer, A., Schuler, A.D., Bedalov, A., Simon, J.A. 2014. Development of Pyrazolone and Isoxazol-5-one Cambinol Analogues as Sirtuin Inhibitors. *Journal of Medicinal Chemistry* 57: 3283-94.

See also: Heltweg, B., Gatbonton, T., Schuler, A.D., Posakony, J., Li, H., Goehle, S., Kollipara, R., Depinho, R.A., Gu, Y., Simon, J.A., Bedalov, A. 2006. Antitumor activity of a small-molecule inhibitor of human silent information regulator 2 enzymes. *Cancer Research* 66:4368-4377.

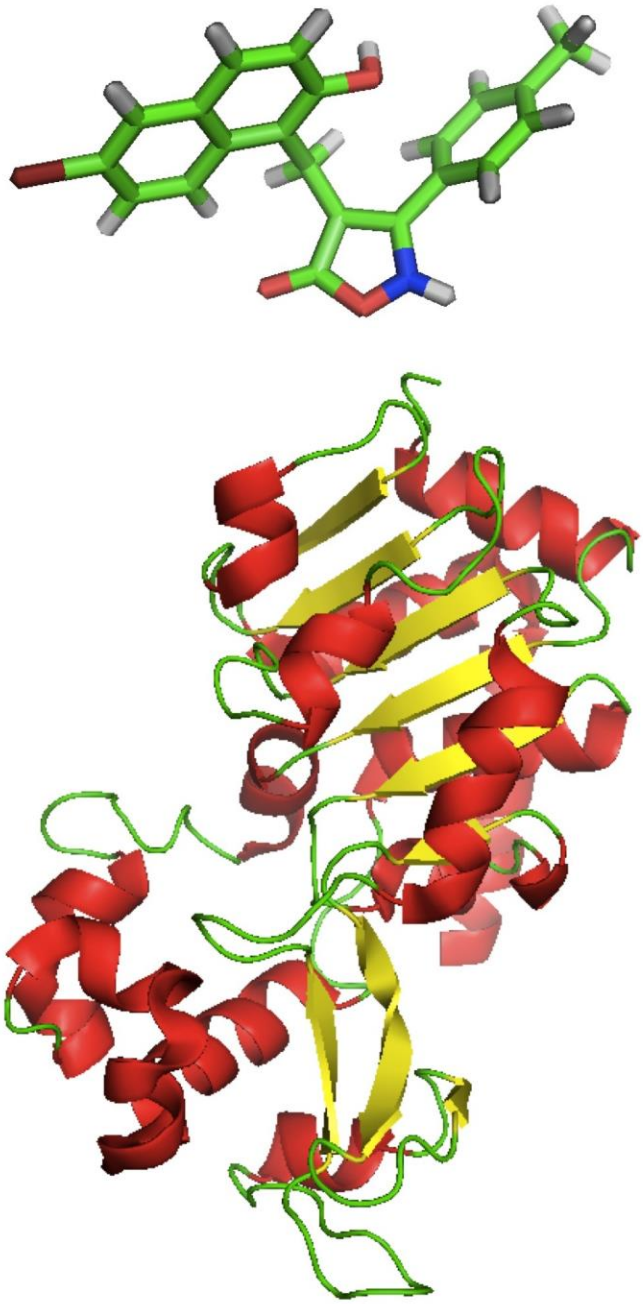

Image provided by Dr. Julian Simon

The structures of the lead compound SM-24 (top) and the SIRT2 protein (bottom). SM-24 selectively inhibits SIRT2 activity and blocks lymphoma cell growth.