Abstract

Sirtuins (SirT1-7) are the human homologues of the NAD^+-dependent histone deacetylase Sir2 from *Saccharomyces cerevisiae* (ySir2). Since the overexpression of Sir2 in yeast increases lifespan, the study of mammalian sirtuins has gained widespread interest. Each sirtuin is characterized by a conserved 275 amino-acid catalytic core domain as well as by unique additional N-terminal and/or C-terminal sequences of variable length. Sirtuins hold promise as potential drug targets for the treatment of a variety of conditions, including cancer, metabolic diseases, diabetes and aging. Although, a number of sirtuin inhibitors as well as activators are known, the inhibitory mechanism of sirtuins is still unknown. In this study, we sought to identify novel inhibitors of SirT1, and we assessed their *in vivo* potential as chemotherapeutic agents. For this purpose, we established a fluorescence-based deacetylation assay using methyl-aminocoumarin-acetyllysine (MAL) as a substrate that is suitable for high-throughput screening. Two compound libraries (500 and 18.000 compounds, respectively) were screened for SirT1-modulating activities, and we identified 14 potential inhibitors and 12 potential activators of SirT1. Of the inhibitors, 9 showed inhibition of SirT1-dependent deacetylation of an acetylated p53 peptide. Interestingly, two of them also inhibited SirT2. Both SirT2 inhibitors were also able to inhibit the p53 deacetylation with IC_{50} values comparable to those determined in the MAL deacetylation assay. Moreover, we observed that the first 220 amino acids of the N-terminal region of SirT1 had an influence on the inhibitory effect of one inhibitor identified here. The potential activators failed to enhance the activity of SirT1 to deacetylate the p53 peptide. Surprisingly, one of them showed strong inhibition of SirT1 in this assay (Hill 2012) as well as inhibition of MAL deacetylation by ySir2 and SirT2. Subsequently, we determined the anticancer potential of the inhibitors identified in this study by different *in vivo* experiments with the lung cancer cell lines A549 and H1299. Three compounds that inhibited cell viability and proliferation of these cancer cells in a dose-dependent manner were pursued in more detail. These three inhibitors induced an additional increase of apoptosis after combined treatment with the chemotherapeutic agent etoposide. We observed that the additional increase of apoptosis mediated by one of theses inhibitors was p53-dependent. In summary, this study has led us to identify one SirT1 inhibitor as well as two SirT2 inhibitors that showed antiproliferation potential and can be developed further for cancer therapy.