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Mutant p53 targeting by the low molecular weight compound STIMA-1

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ABSTRACT

Reactivation of mutant p53 in human tumor cells should induce cell death by apoptosis and thus eliminate the tumor. Several small molecules that reactivate mutant p53 have been identified. Here we show that STIMA-1, a low molecular weight compound with some structural similarities to the previously identified molecule CP-31398, can stimulate mutant p53 DNA binding *in vitro* and induce expression of p53 target proteins and trigger apoptosis in mutant p53-expressing human tumor cells. Human diploid fibroblasts are significantly more resistant to STIMA-1 than mutant or wild type p53-carrying tumor cells. STIMA-1 may provide new insights into possible mechanisms of mutant p53 reactivation and thus facilitate the development of novel anticancer drugs that target mutant p53-carrying tumors.

1. Introduction

The tumor suppressor p53 is expressed at low levels in most cells and tissues under normal conditions. Cellular stress such as DNA damage, oncogene activation, hypoxia and telomere erosion induces p53 protein levels, leading to an array of biological responses, including cell cycle arrest and apoptosis (Vogelstein et al., 2000). p53 exerts its function mainly through transcriptional regulation of specific target genes. Transactivation of p21, Gadd45, and 14-3-3sigma triggers p53-dependent cell cycle arrest, whereas transactivation of Bax, Fas, Noxa and PUMA elicits p53-dependent apoptosis (El-Deiry, 1998; Ryan et al., 2001). p53 is frequently mutated in human tumors, indicating a strong selection for inactivation of the p53 tumor suppressor pathway during tumor evolution (Hainaut and Hollstein, 2000; Olivier et al., 2002).

The high frequency of p53 mutations in human tumors, the high levels of mutant p53 expression in tumors, and the fact that the mutant p53-harbouring tumors often show increased resistance to conventional chemotherapy (Greenblatt et al., 1994; Campling and El-Deiry, 2003) makes p53 an attractive target for novel cancer therapy. Several studies have demonstrated ways to restore normal function to mutant p53

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(Bykov et al., 2003). A C-terminal peptide derived from p53 itself can restore normal function to mutant p53 and induce cell death (Selivanova et al., 1997). CDB3, a rationally designed peptide derived from the p53-binding protein ASPP (Samuels-Lev et al., 2001) binds to the p53 core domain and stabilizes it (Friedler et al., 2002). The small molecule CP-31398 was shown to preserve wild type conformation of p53 upon thermal denaturation, rescue its transactivation capacity, and restore its anti-tumor activity *in vivo* (Foster et al., 1999). CP-31398 also affects wild type p53 (Luu et al., 2002; Takimoto et al., 2002; Wang et al., 2003). However, structural studies did not reveal any binding to p53 (Rippin et al., 2002). PRIMA-1 (Bykov et al., 2002a,b) and MIRA-1 (Bykov et al., 2005a) were identified in a cellular screening of a chemical library from the National Cancer Institute. Both compounds induce mutant p53-dependent apoptosis and restore native conformation, DNA binding, and transcriptional transactivation to mutant p53. PRIMA-1 inhibits tumor growth *in vivo* (Bykov et al., 2002b). The molecular mechanisms for reactivation of mutant p53 by PRIMA-1 and MIRA-1 remain unclear. However, MIRA-1 can potentially react covalently with thiol groups in proteins. Since p53 is known to be subject to redox regulation (Seo et al., 2002), this suggests that modifications of cysteines in the p53 core domain may play a role in mutant p53 rescue.

Despite the successful identification of several mutant p53-targeting small molecules, further screening for new molecular scaffolds using diverse strategies is important to provide a better understanding of the mechanisms of mutant p53 reactivation. Also, the already identified lead molecules may not be suitable for clinical use due to non-specific toxicity or undesirable pharmacodynamic properties.

Compounds structurally related to CP-31398 were shown to possess activity against tumor cells even before the mutant p53-targeting properties of CP-31398 were demonstrated (Jiang et al., 1990). We synthesized and tested a number of 2-styrylquinazolin-4-(3H)-one-related derivatives (Witt and Bergman, 2000, 2001) for mutant p53-dependent growth suppression and identified one, STIMA-1 (2-vinylquinazolin-4-(3H)-one) as the most potent molecule. We show here that both CP-31398 and STIMA-1 (SH group Targeting and Induction of Massive Apoptosis) have similar chemical activity as so called traditional Michael acceptors and that this activity is related to the observed mutant p53-dependent growth suppression. We found that STIMA-1 is more potent than CP-31398 in suppressing growth of mutant p53-expressing tumor cells. Our results provide a better understanding of possible molecular mechanisms behind reactivation of mutant p53 and will hopefully aid the design of novel and more efficient mutant p53-targeting compounds.

2. Results

2.1. Mutant p53-dependent growth suppression by STIMA-1

The structural scaffold of 2-vinylquinazolin-4(3H)-one has been known since 1910 (Bogert et al., 1911). Some of its derivatives have biological activity against cancer cells (Jiang et al., 1990). We noted that these compounds have some resemblance to the newly characterized mutant p53-reactivating compound CP-31398 (Figure 1A) (Foster et al., 1999). To investigate their anti-tumor activity and possible effect on mutant p53, we synthesized a series of 2-styrylquinazolin-4(3H)-one-related derivatives (Witt and Bergman, 2000, 2001) and screened them for mutant p53-dependent inhibition of cell growth in Saos-2 and Saos-2-His273 cells using a WST-1 proliferation assay. Out of 26 molecules, one compound, STIMA-1 (Figure 1A), was identified as having significant mutant p53-dependent growth inhibitory effect. Treatment of H1299-His175 lung carcinoma or Saos-2-His273 osteosarcoma cells with 2 μM STIMA-1 for 96 h and subsequent assessment of cell growth with the WST-1 assay revealed a growth suppression of 41 ± 3.3 for Saos-2-His273 cells and $50.5 \pm 0.6\%$ for H1299-His175 cells, while their p53 null counterparts Saos-2 and H1299 only showed 18.5 ± 0.6 and $2.4 \pm 2.4\%$ growth inhibition, respectively (Figure 1B).

2.2. Chemical reactivity of STIMA-1 and CP-31398

To determine the ability of STIMA-1 and CP-31398 to form adducts with cysteine, 2 μM STIMA-1 and 1 mM CP-31398 were dissolved in PBS and incubated with equimolar amount of N-acetylcysteine (NAC) at 37 °C for 24 h. NAC is a cysteine analog that has an acetyl group instead of an amino group, leaving one SH group as the only target for alkylation. Samples were analyzed by reverse phase HPLC. Figure 2A shows that incubation of both STIMA-1 and CP-31398 with NAC resulted in the formation of new peaks on HPLC. Around 97% of STIMA-1 and 20% of CP-31398 reacted with NAC as judged by

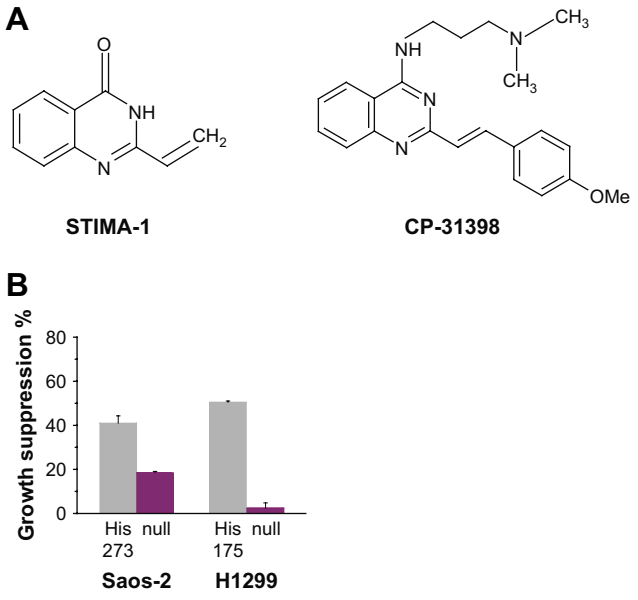

Figure 1 – (A) Chemical structure of STIMA-1 and CP-31398. (B) STIMA-1-induces mutant p53-dependent growth suppression in Saos-2-His273 and H1299-His175 cells. Cell growth was assessed using the WST-1 proliferation agent. Data represent mean ± standard error ($n = 3$).