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Thioredoxin reductase as a target enzyme for electrophilic anticancer drugs

AKADEMISK AVHANDLING

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ABSTRACT

Induced production of reactive oxygen species (ROS) is a common attribute of most cancer cells. One strategy for cancer cells to maneuver the increased and potentially toxic levels of ROS is to induce the expression of cellular antioxidants and redox regulators, such as the thioredoxin (Trx) system. The Trx system consists of Trx and the NADPH-dependent thioredoxin reductase (TrxR protein/*Txnrd1* gene). TrxR reduces Trx, which subsequently reduces disulfides in various proteins and supplies ribonucleotide reductase with electrons for DNA synthesis. Mammalian TrxRs have wide substrate specificity, also reducing other targets than Trx. Cytosolic Trx1 and TrxR1 are induced upon oxidative stress and both have proven to be overexpressed in many tumors. They are therefore proposed as potential targets for anticancer therapy. TrxR is a selenoprotein and contains selenium in the form of selenocysteine (Sec). The Sec residue is mostly de-protonated at physiological pH and is highly nucleophilic, thus being easily targeted by electrophilic drugs.

The aim of this thesis was to address the role of TrxR1 as a potential drug target for anticancer therapy and evaluate its importance for side effects associated with the widely used anticancer drug cisplatin (cDDP).

This thesis reports that RITA, a compound shown to induce p53 dependent cell-death by interacting and restoring p53 activity, caused inhibition of TrxR1. Cell culture experiments showed that RITA induced a 130 kDa covalently linked TrxR1-dimer, in a p53 dependent fashion. Furthermore, red wine, rich of polyphenols and flavonoids, was also shown to efficiently inhibit TrxR activity and to be highly toxic to various cancer cell lines.

Transient TrxR1 knockdown in a lung carcinoma cell line lowered the TrxR activity by 90% and caused increased sensitivity towards menadione and 1-chloro-2,4-dinitrobenzene. TrxR1 knockdown cells were, however, more resistant towards cDDP. Depleting the glutathione (GSH) levels in knockdown cells had no effect on cell growth, suggesting that the remaining TrxR activity still was enough to sustain Trx function. Recent experiments in mice showed that normal replication of hepatocytes required either one functional copy of the *Txnrd1* gene or a functional GSH system, agreeing with the previous interpretation.

cDDP treatment is associated with side effects such as ototoxicity and nephrotoxicity. cDDP inhibits TrxR1 and cDDP-derivatized enzyme species have previously been shown to gain a pro-oxidant role in the cells. Data on cDDP-triggered nephrotoxicity in mice presented herein suggest that the degree of kidney damage is influenced by the TrxR status in both liver and kidney. Decreased TrxR activity in liver was associated with more renal damage, while high TrxR expression in kidney correlated with increased kidney toxicity. Pharmacokinetic studies on cDDP and oxaliplatin (Oxa) in guinea pig, showed that the cochlear uptake of cDDP was significantly higher than for Oxa, thus explaining why Oxa only rarely causes ototoxicity. Using a cancer cell line it was also shown that cDDP, but not Oxa, induced cell death which was dependent on calcium and superoxide levels and caused TrxR inhibition.

In summary, this thesis shows that TrxR1 is an anticancer drug target that can have an important impact on the outcome of chemotherapy and its associated side effects.