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# **CYTOCHROME P450 2W1 (CYP2W1) AS A NOVEL DRUG TARGET IN COLON CANCER THERAPY**

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentlig  
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av

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## ABSTRACT

Cytochrome P450 2W1 (CYP2W1), is a monooxygenase enzyme endogenously expressed in fetal colon and normally silenced after birth. In adult life, the expression of *CYP2W1* occurs exclusively in tumor cells yielding high amounts of the enzyme in 30% of human colon cancers. Remaining the third most commonly diagnosed malignancy in the world, the colon cancer requires new chemotherapeutic strategies that would provide higher selectivity towards transformed cells. The unique confinement of CYP2W1 to tumor tissue opens the possibility for its targeting in colon cancer therapy. The studies in this thesis have focused on characterization of the CYP2W1 enzyme and the development of novel anticancer prodrugs designed for CYP2W1-specific activation.

In cancer tissues and transfected cells CYP2W1 gives multiple immunoreactive bands, suggesting that the protein might be subjected to posttranslational modifications. Our *in vitro* and *in vivo* studies reveal that CYP2W1 undergoes glycosylation at Asn177, which is enabled by the unique inverted topology of the protein in the ER membrane. Regardless of the reversed emplacement, CYP2W1 retains its catalytic function in cell systems and is capable of converting inactive substrates to potent cytotoxic species, demonstrating the potential for prodrug activation.

With the goal to develop antitumor prodrugs for specific activation by CYP2W1, we have employed a library of novel duocarmycin-based compounds and identified ICT2705 and ICT2706 as the first molecules whose cytotoxic activity is dependent on CYP2W1 enzyme. Our studies reveal the results of the first attempt of CYP2W1 targeting in cancer, both *in vitro* and *in vivo*. We show that the colon cancer cell lines expressing CYP2W1 suffer substantial DNA damage and rapid loss of viability following incubation with ICT2705 and ICT2706. Moreover, we demonstrate that the CYP2W1-positive human xenografts undergo complete growth inhibition in mice dosed with ICT2706, with no apparent deleterious effects detected in any of the treated animals. In addition, we present *in vitro* evidence that the potent cytotoxic effect is most likely propagated by a bystander killing mechanism.

Polymorphisms in *CYP2W1* gene yield two alleles of significant frequencies, designated *CYP2W1*\*2 and *CYP2W1*\*6. The resulting variant enzymes carry missense mutations that might affect the therapeutic outcome of prodrug targeting as well as the course of the metastatic disease. A recent study has associated *CYP2W1*\*2 with a decreased risk for colon cancer, suggesting the altered catalytic activity of this enzyme variant. Using the novel duocarmycin substrates, we were able to evaluate the catalytical properties of the variant enzymes for the first time. In contrast to previous findings, our studies show comparable catalytic capacity for all CYP2W1 enzymes, and no association between *CYP2W1*\*2 genotype and colon cancer risk based on 10-fold larger patient cohorts.

In conclusion, we have found that CYP2W1 is a glycosylated enzyme with reversed ER topology, whose catalytic activity is sustained in an intact cell system and unaffected by the most frequent polymorphic changes. We have developed first prodrugs for CYP2W1 targeting in cancer and demonstrated their antitumor efficacy in a preclinical setting. The work presented herein provides the basis for a novel therapeutic approach in colon cancer chemotherapy.