Biomedical Data Mining

Extended Abstract

Recombinant expression of cytochrome P450-2D6 and its application in tamoxifen metabolism

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Abstract

Introduction: Breast cancer is the leading cause of most fatal incidences in women worldwide. It is mostly induced by hormone oestrogen which stimulates the DNA to proliferate cells into cancerous cells. Tamoxifen is administered as a pro-drug for both treatment and prevention of breast cancer in women and men who are oestrogen positive. Tamoxifen metabolites function by binding and competing with oestrogen for binding to oestrogen receptors thus blocking the development of cancerous cells. Cytochrome p450-2D6 (CYP2D6) is one of the main enzymes in the tamoxifen metabolism. There is an inter-individual difference in response to tamoxifen treatment due to polymorphism in this enzyme. Therefore, there is a need to diagnose whether patients taking tamoxifen are able to metabolize the drug. The currently used assay for patient's tamoxifen metabolism have limitations including non-specific, time consuming and cost effective.

Aim: This study aimed to develop a CYP2D6 based electrochemical biosensor which will test the metabolism of tamoxifen in breast cancer patients.

Methods: Physico-chemical parameters of CYP2D6 were determined to pave way for CYP2D6 gene amplification, cloning into pTrcHis TOPO vector, over-expression in E.coli followed by purification, in order to obtain an active recombinant CYP2D6. Tamoxifen metabolism by CYP2D6 was assayed using UV-Visible and emission spectra and validated by electrochemical techniques using CYP2D6 based-biosensor.

Results and Discussion: CYP2D6 is a 50.05397 kDa insoluble trans-membrane protein that is slightly acidic in nature (pI = 6.21). The CYP2D6 gene was successfully amplified as characterized by a 1.375bp band and subsequently cloned into pTrcHis TOPO vector. The protein was overexpressed in TOP10 E. coli cells, extracted and purified under denatured condition since it was expressed in the inclusion bodies. The protein was successfully refolded to its active form as determined by the P450 GLO CYP2D6 assay. CYP2D6 is characterized by a Soret band at 215 nm (UV-Vis) and 425 nm (emission spectra). Electrochemical assays indicated that the enzyme is active and has the ability to metabolize tamoxifen into its active forms at a potential of 0.6 V. Therefore, these results are adequate to be applied in the development of CYP2D6 based sensor for tamoxifen metabolism during breast cancer therapy.

Conclusion

Breast cancer is the most common malignancy among women. Its lifetime risk amounts to a total of 10%, and approximately 15–20% of all breast cancers are associated with the occurrence of familial breast and/or ovarian cancer. During the past two decades, various high- and low-risk cancer susceptibility genes have been detected, including high-risk susceptibility genes such as breast cancer gene 1 (*BRCA1*) and breast cancer gene 2 (*BRCA2*). Although the role of inheritance in breast cancer carcinogenesis is well established, an emerging area of research is pharmacogenetics, a field that studies the role of genetic inheritance in individual variation in drug response and toxicity.

Recently, genetic and drug-induced variation in the phase I drug-metabolizing enzyme cytochrome *P*450 2D6 (CYP2D6) has emerged as an important contributor to the interindividual

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variability in response after the administration of tamoxifen.

For three decades, tamoxifen has been a standard endocrine therapy for the treatment of ER-positive breast cancer. When administered to women with ER-positive breast cancer for 5 years after surgery, tamoxifen almost halves the annual recurrence rate and reduces the breast cancer mortality rate by one-third in both pre- and post-menopausal women. Tamoxifen's continued importance is reflected by its status as the only hormonal agent approved by the US Food and Drug Administration (FDA) for the prevention of breast cancer, the treatment of ductal carcinoma *in situ*, and the treatment of pre-menopausal breast cancer.

Although tamoxifen is still the accepted standard of care for pre-menopausal breast cancer, aromatase inhibitors (AIs) are now an accepted therapy for post-menopausal breast cancer. In this setting, there are two accepted treatment algorithms: an AI for 5 years or tamoxifen for 2–3 years followed by an AI for 2–3 years. Compared with 5 years of tamoxifen, administration of an AI for 5 years reduces the risk of a disease event by 13%, but does not prolong survival. In contrast, sequencing of hormonal therapy with tamoxifen for 2–3 years followed by an AI reduces the risk of a disease event by 40% and prolongs survival compared with 5 years of tamoxifen. For this reason, tamoxifen is still commonly employed for treatment of post-menopausal breast cancer.

When more than one effective option exists for treating a given disease, there is much interest in identifying biomarkers which can reproducibly identify patients that should preferentially receive or be excluded from a given therapy. Notably, tumors or somatic markers (*e.g.*, ER, HER-2) are not predictive of a preferential response to breast cancer endocrine therapy (*e.g.*, tamoxifen vs. aromatase inhibitor). In this study, we review the pharmacology of tamoxifen biotransformation, the importance of *CYP2D6* pharmacogenetics accounting for the variability in exposure to the active tamoxifen metabolites, and evidence to suggest that CYP2D6 can be used as a predictive marker for the individualization of endocrine therapy.

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