Breast Cancer Online
www.bco.org

*Breast Cancer Online (www.bco.org)* 2004; **7**(10),
doi:10.1017/S1470903104002962

ISSN 1470-9031

*Journals Club*

# Review of: Identification of a novel mammary-restricted cytochrome P450, CYP4Z1, with overexpression in breast carcinoma

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**Citation of original article:**
M. A. Rieger, R. Ebner, D. R. Bell, A. Kiessling, J. Rohayem, M. Schmitz, A. Temme, E. P. Rieber, B. Weigle. ***Cancer Research*** 2004 Apr 1; **64**(7): 2357–64.

**Abstract of the original article***
By screening a transcriptome database for expressed sequence tags that are specifically expressed in mammary gland and breast carcinoma, we identified a new human cytochrome P450 (CYP), termed CYP4Z1. The cDNA was cloned from the breast carcinoma line SK-BR-3 and codes for a protein of 505 amino acids. Moreover, a transcribed pseudogene CYP4Z2P that codes for a truncated CYP protein (340 amino acids) with 96% identity to CYP4Z1 was found in SK-BR-3. CYP4Z1 and CYP4Z2P genes consisting of 12 exons are localized in head-to-head orientation on chromosome 1p33. Tissue-specific expression was investigated using real-time reverse transcription PCR with normalized cDNA from 18 different human tissues. CYP4Z1 mRNA was preferentially detected in breast carcinoma tissue and mammary gland, whereas only marginal expression was found in all other tested tissues. Investigation of cDNA pairs from tumour/normal tissues obtained from 241 patients, including 50 breast carcinomas, confirmed the breast-restricted expression and showed a clear overexpression in 52% of breast cancer samples. The expression profile of CYP4Z2P was similar to that of CYP4Z1 with preference in breast carcinoma and mammary gland but a lower expression level in general. Immunoblot analyses with a specific antiserum for CYP4Z1 clearly demonstrated protein expression in mammary gland and breast carcinoma tissue specimens as well as in CYP4Z1-transduced cell lines. Confocal laser-scanning microscopy of MCF-7 cells transfected with a fluorescent fusion protein CYP4Z1-enhanced green fluorescent protein and a subcellular fractionation showed localization to the endoplasmic reticulum as an integral membrane protein concordant for microsomal CYP enzymes.

## Review

The identification of breast cancer-associated antigens may be useful in early diagnosis of mammary carcinoma. In this regard, the novel cytochrome P450 (CYP) gene CYP4Z1 detected by Reiger *et al.* in mammary gland and breast carcinoma is of great interest [1]. Following bioinformatic analysis of a transcriptome database the authors isolated the new CYP cDNA from SK-BR-3 cells. A transcribed pseudogene CYP4Z2P which encodes a truncated CYP analogous to CYP4Z1 was also isolated.

CYPs hemoproteins are encoded by a superfamily of closely related genes and mediate the oxidative biotransformation of lipophilic compounds. CYPs oxidize foreign chemicals, including drugs and carcinogens, and endobiotics, such as steroids and fatty acids, to more polar products that are usually more readily eliminated. At present there are more than 55

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* http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15059886

Publication date 27/10/04
BCO/296/2004/JC

different human CYP genes, excluding pseudogenes and variant alleles, that are subdivided into families and subfamilies on the basis of amino acid sequence similarity [2]. Most xenobiotic-oxidizing CYPs are found in families 1–3, whereas CYPs from family 4 and higher usually mediate endobiotic or vitamin oxidation. In most instances CYPs deactivate lipophilic chemicals, but there are many examples of substrates that exert pharmacological or toxicological effects after oxidation by CYPs. The mutagenicity of polycyclic aromatic hydrocarbons and arylamines is increased by the action of CYPs that generate reactive products that may then interact with cellular DNA. Previous studies have evaluated whether the expression of such CYPs in tumours may be linked to tumourigenesis [3–5]. Knowledge of the biological functions and the cellular regulation of CYPs that are overexpressed in tumours may provide insight into the underlying disease pathology.

In addition to the identification of this new gene, a major finding of the study of Reiger *et al.* was that the median expression of CYP4Z1 mRNA in breast carcinoma samples was at least twice that in normal breast tissue. However, the proportion of samples of tumour and normal that were positive for expression was similar. No major differences in expression were noted between the stages or types of the breast tumour although, as suggested by the authors, the analysis of further well-characterized tumour samples is now warranted. Very low-level expression of CYP4Z1 was detected in ovary, lung, thyroid and prostate. Thus, CYP4Z1 appears to be preferentially expressed in mammary gland and to be overexpressed in cancerous tissue. Other aspects of CYP4Z1 were also elaborated in the study. Like other CYPs, the subcellular location of CYP4Z1 is the endoplasmic reticulum. The CYP4Z1 gene and the pseudogene CYP4Z2P are both located on chromosome 1p33 in close proximity to the related CYP genes 4A11, 4B1 and 4X1. CYP4Z2P itself probably arose from an inverted duplication event and the truncated protein is non-functional because it lacks a substrate-binding region. Low-level transcription of the pseudogene could be due to mRNA instability or the loss of gene regulatory sequences.

That the new CYP is a member of the CYP4 family could be very significant. Other members of this family have important homeostatic roles. CYP4A11 is the human fatty acid ω-hydroxylase that is expressed in a wide range of tissues and is about 90% similar to CYP4Z1 at the amino acid level, with 50% sequence alignment. The terminal hydroxyarachidonate products formed by CYP4A11 modulate local blood pressure [6]. CYP4B1 oxidizes the procarcinogens 2-aminofluorene and 2-ipomeanol to toxic products and its high-level expression in organs of the gastrointestinal tract could be a risk factor for tumourigenesis [7]. CYP4F subfamily enzymes mediate the oxidation of the proinflammatory leukotriene B4 and may therefore regulate inflammatory processes [8]. Some CYP4 enzymes are preferentially expressed in certain cells or organs. Thus, CYP4F3 appears restricted to polymorphonuclear leukocytes, CYP4F12 is found in the small intestine and the recently described CYP4X1 is expressed in brain [8,9]. Variants of CYP4V1 have been implicated in Bietti crystalline corneoretinal dystrophy [10], which is consistent with the assertion that defective CYP alleles may be determinants of disease processes. It has been suggested that the wild-type allele may perform critical fatty acid oxidations in the eye.

To appreciate the cellular function of CYP4Z1 further experimentation is required. Heterologous expression of the protein would be useful in evaluating substrate oxidation behaviour, but this should be complemented with studies in fractions from breast cancer tissue. The combined use of these approaches will minimize artefacts due to the low substrate specificities of CYPs when studied in isolation.

Information on the regulation of CYP4Z1 could be valuable in accounting for the apparent overexpression of the gene in breast carcinoma. Other CYPs have been detected at very low level in breast tissue. Thus, the study of Hellmold *et al*. [11] is one of several that detected low levels of CYPs from families 1–3, CYP4A11 and CYP19 (aromatase; estrogen synthase) by reverse transcriptase polymerase chain reaction (RT-PCR) and western blotting in mammaplasty samples, consistent with the assertion that breast tissue has drug and xenobiotic-oxidizing capacity. Another study reported the expression of CYP4B1 in breast tissue; these workers did not detect differences in CYP expression between non-tumour and tumour tissues [12]. The excellent study of Larsen *et al.* correlated CYP1B1 overexpression in breast tumours with increased function, including activation of polycyclic aromatic hydrocarbons [5].

As pointed out by Reiger *et al.*, the identification of tumour-associated antigens may be of diagnostic utility especially given that the number of established breast cancer-specific antigens is limited. Moreover, as further information on the function and regulation of CYP4Z1 emerges, important insights may be obtained into the role of this enzyme in normal breast function and the impact of its apparent overexpression in tumour tissue.

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