Differential expression of CYP1A1 and CYP1B1 in human breast cancer

JUDITH A MCKAY*, GRAEME I MURRAY*, AK AH-SEE*, WILLIAM F GREENLEE†, CRAIG B MARCUS*, M DANNY BURKE‡ AND WILLIAM T MELVIN§.

Departments of *Pathology, †Surgery, ‡Biomedical Sciences and §Molecular and Cell Biology, University of Aberdeen, Aberdeen, AB9 2ZD; †Department of Pharmacology and Toxicology, Purdue University, West Lafayette, USA.

The cytochromes P450 (P450) are a multi-gene superfamily of constitutive and inducible enzymes and are involved in the oxidative metabolism of a diverse range of xenobiotics including carcinogens, therapeutic drugs and several groups of biologically active endogenous compounds such as steroid hormones and fatty acids. The main families of P450 involved in xenobiotic metabolism each consist of several individual forms with specific regulatory mechanisms and substrate specificities. Until recently the CYPl gene family which is the main P450 family involved in the metabolism of polycyclic aromatic compounds and arylamines and is regulated by the Ah receptor complex, was considered to consist of one subfamily consisting of two distinct but closely homologous members, CYP 1A1 and CYP 1A2. CYP 1A1 is an inducible P450 found primarily in extrahepatic tissues while CYP 1A2 is constitutively expressed in liver. Recently a new dioxin-inducible CYPl P450 subfamily has been identified, containing one form to date, CYPlB1[1]. In the current study both CYPlA1 and CYPlB1 mRNA were shown to be expressed in breast tumours, with CYPlB1 mRNA being the most frequently expressed.

RT-PCR was performed using RNA isolated from primary breast cancers. cDNA was synthesised from the isolated RNA using oligo (dT)$_{18}$. The CYP1A primers recognised sequences common to both CYPlA1 and CYPlA2 and each product contained a different Rsal digestion site allowing distinction between CYPlA1 and CYPlA2 sequences by gel electrophoresis after Rsal digestion. The CYPlB1 primers recognised sequences at the 3' noncoding region of the CYPlB1 gene. The PCR products were analysed by agarose gel electrophoresis and sequencing to confirm the specificity of the reaction. Immunoblotting was performed using an enhanced chemiluminescence technique [2].

Of the 40 breast cancers studied by RT-PCR 29 of the tumours (73%) amplified for CYPlB1 and sequencing of the purified PCR product showed identity with CYPlB1. RT-PCR for CYPlA showed that 10 of the 40 tumours (25%) amplified for CYPlA and direct sequencing of the undigested CYPlA PCR product showed identity with CYPlA1. Following digestion of the PCR product with Rsal, two bands of 535bp and 114bp respectively were identified, consistent with the digestion products of CYPlA1. None of the tumours, however, showed evidence of expression of CYPlA2 mRNA. There was only one tumour which expressed CYPlA1 without co-expressing CYPlB1 mRNA while 10 of the tumours did not express any member of the CYPl family. Immunoblotting of breast tumours showed the presence of CYPlB1 protein.

The expression of individual forms of the CYPl gene family in breast cancer has been investigated. It had been widely thought that the CYPl gene family consisted of only one subfamily, containing two highly homologous members, CYP 1A1 and CYP 1A2. Recently, however, a new member of the CYPl family, CYPlB1, belonging to a new subfamily, was identified in dioxin induced human keratinocytes and showed approximately 40% identity with both CYPlA1 and CYPlA2. In the current study both CYPlA1 and CYPlB1 mRNA were shown to be expressed in breast tumours, with CYPlB1 mRNA being the most frequently expressed.

Both CYPlA1 and CYPlB1 have been shown to be regulated by compounds which act at the Ah receptor. However, this study clearly shows differential expression of CYPlA1 and CYPlB1, with CYPlB1 being the most frequently expressed member of the CYPl gene family in breast cancer. The expression of CYPlB1 in breast cancer is likely to have important metabolic consequences for the tumour cells, e.g. with respect to oestrogen metabolism as breast cancer is an oestrogen dependent tumour. The differential expression of CYPlA1 and CYPlB1 indicate different regulatory mechanisms controlling CYPlA1 and CYPlB1 expression in breast cancer.

This research has been supported by The Scottish Office Home and Health Department
