
Cytochrome P450s and chemoprevention
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Abstract
The cytochrome P450 mono-oxygenase system represents a major defence against chemical challenge from the environment, constituting part of an adaptive response mounted by an organism following exposure to harmful agents. Cytochrome P450s are also able to catalyse the activation of compounds to toxic products, and participate in a variety of essential 'housekeeping' functions, such as biosynthesis of steroid hormones and fatty acid oxidation. It is clear that the modulation of expression of these enzymes can have a significant effect on chemical toxicity, carcinogenicity and mutagenicity. The concept of cancer chemoprevention, i.e. the administration of a (non-toxic) chemical or dietary component in order to prevent neoplastic disease or to inhibit its progression, is an attractive one. Despite this, relatively little work has been done to characterize the ability of putative chemopreventive agents to modulate P450 expression, or to understand the interaction between P450s and chemopreventive agents. Before chemopreventive treatment can become a reality, it is essential that this complex issue is addressed; for instance, it is likely that any single chemopreventive agent will induce more than one P450 isoenzyme, and while altered expression of a particular P450 may attenuate the effects of one toxic agent, the effects of others might well be potentiated. Our laboratory has created a transgenic mouse line in which the rat CYPlAl promoter drives expression of the β-galactosidase gene. These mice can be used to define which compounds act via the Ah receptor, in which tissues, and at which stage of development. We are currently developing another mouse line in which β-galactosidase expression is controlled by the mouse GstA1 promoter, allowing us to define the role of the antioxidant responsive element in the action of chemopreventive agents. Finally, using cre-loxP transgenic technology, we have generated a mouse line in which P450 reductase can be deleted in a conditional, i.e. tissue-specific, manner, permitting us to investigate the role of P450s in chemoprevention in a more defined manner.

Chemopreventive agents and drug-metabolizing enzymes
The significant part played by environmental factors in determining cancer susceptibility indicates that, potentially, it may be possible to prevent or inhibit the carcinogenic process [1]. There are a number of ways in which this goal could be achieved, and among these, the modulation of host defences against environmental chemicals promises much. A number of complex enzyme systems have evolved to protect against the toxic effects of environmental chemicals, and these may act in a variety of ways. In mammals, hepatic metabolism is the major factor in determining the circulating concentration of toxin, mutagen or carcinogen, although significant metabolic potential also exists at extra-hepatic sites, i.e. the lung, gut and skin. Other factors that will contribute to cellular sensitivity include transport of compounds into and out of the cell, metabolism within the cell itself, and the ability of the cell to repair DNA damage.

Central to the defensive armamentarium against toxic chemicals is xenobiotic metabolism [2,3]; historically, enzymes involved in foreign compound metabolism have been considered to exist in two groups: Phase I and Phase II enzymes. The former, which includes cytochromes P450, metabolize lipophilic compounds to more polar products. The products of Phase I metabolism are then acted upon by Phase II enzymes, which include glutathione S-transferases (GSTs), sulphotransferases, N-acetyltransferases and UDP-glucuronyltransferases, further increasing their polarity and assisting in their excretion. It is clear that both P450s and GST play a pivotal role in determining cellular sensitivity to environmental chemicals, and that modulation of the ex-

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Abbreviations used: ARE, antioxidant responsive element; GST, glutathione S-transferase.
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pression of these enzymes by chemopreventive agents is an important part of their mechanism of action [4]. Chemopreventive agents have been divided into two broad groups, depending on whether or not the compound is perceived to act before, or after, the mutagenic step(s) of the carcinogenic process [4]. Compounds that act post-mutagenesis have been termed ‘suppressing’ agents, and these include retinoids, indoles, carotenoids and non-steroidal, anti-inflammatory drugs. Such compounds operate in a diverse manner, e.g. by enhancing apoptosis, or inhibiting the activation of oncogenes. Compounds that prevent mutagenesis have been termed ‘blocking’ agents, and these include flavones, coumarins, phenols and terpenes [5,6]. Blocking agents can be further sub-divided on the basis of their ability to regulate the expression of Phase I and Phase II enzymes. Bifunctional inducers are capable of inducing both Phase I and Phase II enzymes, whereas monofunctional inducers only increase the expression of Phase II enzymes. A further group of compounds has been described, which induce Phase II enzymes but inhibit the expression of Phase I enzymes, and have been called dual-acting agents [5–8] (Figure 1).

Cytochromes P450 have evolved as an adaptive response system to environmental stress: challenged by a specific chemical, the cell responds by activating transcription of one or more P450s, resulting in the metabolism and excretion of the compound. Despite the acknowledged importance of P450-based metabolic pathways in determining sensitivity to environmental agents, relatively little is known about the effects of chemopreventive agents on the expression of these enzymes [1,5–8]. This is a crucial issue, since metabolism by P450s can lead not only to an increased rate of chemical deactivation, i.e. detoxification, but, under some circumstances, can result in chemical activation and the generation of toxic metabolites. Thus it is clear that, while the induction of one particular P450 might be beneficial in terms of protection against one particular compound, it might potentiate the deleterious effects of other agents to which an individual has also been exposed. This situation is further complicated by the fact that P450s are often expressed in a tissue-specific manner, and that, furthermore, chemopreventive agents may also exhibit tissue-specific effects.

**Modulation of cytochrome P450 expression by chemopreventive agents**

In an attempt to unravel the effects of chemopreventive agents on P450 expression, male Fisher 344 rats were treated with a variety of chemopreventive agents, as described in the legend to Figure 2. Following treatment, tissues were removed, the microsomal fraction prepared and Western blotting was performed with P450 antisera, as described previously [9] (Figure 2). In the liver, the panel of chemopreventive agents had

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**Figure 1**

*Overview of chemopreventive agents and their action on drug-metabolizing enzymes*

+ve and -ve has a potentiating or an attenuating effect respectively.

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differing effects on P450s from different subfamilies. Ethoxyquin, hexachlorobenzene and indole-3-carbinol all significantly induced CYP1A1/2, CYP2B1 and CYP3A1 proteins relative to the animals on the control diets, whereas these compounds had much less of an effect on CYP4A1. In contrast, benzylisothiocyanate strongly increased the expression of CYP4A1 in liver, while having no effect on protein levels in the other CYP subfamilies. Diallylsulphide, while having no effect on CYP1A1/2 expression, strongly induced CYP2B1 and CYP3A1, and appeared to suppress expression of CYP4A1.

In the kidney, modulation of P450 expression by chemopreventive agents was much less dramatic:

In the lung, most of the chemopreventive agents used induced expression of CYP1A1, although the extent of induction varied with the compound, with the greatest potentiation being observed with ethoxyquin and indole-3-carbinol, while p-methoxyphenol was the poorest inducer of this P450 in the rat lung. A similar situation was observed with CYP2B1; the majority of compounds included in this study suppressed levels of this P450, with benzylisothiocyanate and coumarin completely abolishing expression. Reminiscent of CYP1A1 expression in the kidney, only indole-3-carbinol showed any effect on CYP4A1 in the lung.

The data in Figure 2 illustrate the complex effects elicited on the expression of P450 isoforms in different tissues in the rat. A single chemopreventive agent can have opposing effects on the same P450 in different organs; for instance, diallylsulphide strongly induces expression of CYP2B1 in liver, while suppressing expression of the same protein in the lung. Similarly, benzylisothiocyanate causes the production of high levels of CYP4A1 protein in rat liver, while abolishing expression in the lung. Thus, although the inductive effects of any particular chemopreventive agent may be beneficial in one organ, opposing effects elicited by the same compound in other organs may offset this. Even within one tissue, a single chemopreventive agent can have opposite, and potentially damaging effects; e.g. indole-3-carbinol induces the expression of CYP1A1 and CYP4A1, but suppresses CYP3A1 and CYP2B1 expression in kidney and lung respectively.

In summary, chemopreventive agents are capable of inducing significant changes in the expression of P450 proteins, not only in the liver, but also in lung and kidney. Whether or not these changes are beneficial depends on the overall balance of P450 expression in the organism as a whole, as well as the levels of other Phase I drug-metabolizing enzymes and Phase II enzymes, all of which can be affected by chemopreventive agents. It is clear that much more work needs to be done to explore more fully the action of chemopreventive agents, and to understand in more detail how these compounds regulate the expres-
sion not only of P450s, but also other drug-metabolizing enzymes, such as GST.

**Transgenic models of gene expression**

One way to investigate further how environmental chemicals alter gene expression is to utilize transgenic technology [10]. We have previously reported a transgenic mouse line, in which the rat CYP1A1 promoter controls expression of β-galactosidase, allowing direct detection of CYP1A1 promoter activity (and thus Ah receptor function) in a spatio-temporal manner [11]. In an extension of this principle, we are currently developing a transgenic mouse line in which the mouse GstA1 promoter, containing an antioxidant responsive element (ARE), drives β-galactosidase expression (Figure 3A). The ARE was first identified in the rat GSTA2 gene [12]. The core consensus sequence has since been refined to 5'-GTGACNNNGC-3', and this element has been found in the promoter of detoxification enzymes induced by chemopreventive agents, e.g. haem oxygenase 1 (HO-1), NAD(P)H:quinone oxidoreductase (NQO1) and γ-glutamyl cysteine synthetase (the heavy subunit) [13]. A transgenic mouse line in which a reporter gene is linked to a promoter containing an ARE will allow us to study in greater detail: (i) which compounds act as inducers via this element, (ii) in which tissues, and (iii) at which stage in development. It will also permit characterization of those factors involved in binding to the ARE, such as Nrf2 [14,15].

Cytochrome P450 reductase is the terminal electron acceptor in an electron-transport chain, transferring reducing equivalents from NADPH to cytochromes P450. Deletion of this gene might be expected to be lethal, although it is possible that alternative pathways might support a certain degree of electron transport, i.e. cytochrome b5 reductase and adrenodoxin reductase. If it were possible to delete cytochrome P450 reductase in a localized manner, i.e. in certain tissues, or at certain stages of development, then valuable information might be gained concerning the role of P450s in the action of chemopreventive agents. This possibility is currently being pursued by the creation of transgenic mice in which loxP sites [16,17] flank a key region of the reductase gene. These small (40-bp) palindromic repeats act as targets for Cre recombinase, which excises DNA between loxP sites, thus rendering the reductase gene inactive. The key to this project is the delivery of the Cre recombinase in a tissue-specific manner. We are at present utilizing an adenovirus-based system [18], which, although mainly effective in the liver, will also delete P450 reductase more generally, and a mouse line in which Cre recombinase is under the control of the albumin promoter, resulting in hepatic expression, and thus liver-specific inactivation, of the reductase gene [19] (Figure 3B).

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**Figure 3**

Transgenic approaches to the investigation of gene regulation

(A) Construct for the GSTAI/β-galactosidase transgenic mouse line (mGSTAI-βGal). Ex1 and Ex2, exons 1 and 2 respectively. (B) Construct for the conditional deletion of cytochrome P450 reductase.

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Concluding comments
The modulation of expression of drug-metabolizing enzymes by chemopreventive agents, while playing a pivotal part in their mechanism of action, is a complex affair. In order to unravel this complexity, it will be necessary to understand in greater detail not only how such compounds induce/suppress gene expression, but also in which tissues the modulation occurs, and at what stage in an organism's development. Transgenic technology has a vital role to play in answering these questions, which will undoubtedly assist in the realization of the anti-carcinogenic potential of chemopreventive compounds.

References

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