# S-6

## CHARACTERIZATION OF RECEPTOR-MEDIATED MECHANISMS OF CHEMICAL CARCINOGENESIS

Russell C. Cattley
Chemical Industry Institute of Toxicology
Research Triangle Park, NC, USA

### Introduction

Chemicals that induce cancer in laboratory animal studies are suspected of posing increased risk of cancer in humans. While the precise mechanism of action for any chemical carcinogen is characterized by complexity and uncertainty, identifying a conceptual framework to characterize the general mode of action for a variety of chemicals has been possible. One primary mode of carcinogen action is genotoxicity (also known as mutagenicity), wherein a chemical or its metabolite interacts with genomic DNA, resulting in mutations in critical oncogenes or tumor suppressor genes. For those chemicals that lack the ability to interact with DNA, a non-genotoxic mode of action can be proposed. However, there is probably a variety of molecular and cellular targets involved in carcinogenesis by nongenotoxic agents. One of these modes of action involves altered binding and activation of cellular receptors. This presentation will address the concept of receptor-mediated carcinogenesis and review recent advances in understanding the mechanism of a unique class of receptor-mediated carcinogens, the peroxisome proliferators.

### Receptor-mediated carcinogenesis

One of the current challenges in toxicology is characterization of the pathogenesis of chemical carcinogenesis that is mediated by changes in receptor activation. A heightened awareness and interest in this mode of action are probably reflective of several trends, including (1) the general reluctance to develop or use chemicals that directly damage DNA, (2) the impact of new molecular biological capabilities, leading to rapid identification of new receptors, and (3) attempts to discover chemicals that interact with receptors as a rational approach to drug discovery.

A receptor is a cell constituent that has the dual property of binding agonists with high affinity and specificity and triggering a cellular response as the result of conformational change. An altered receptor function could mediate the carcinogenic response to a chemical in a variety of ways. However, two generic mechanisms are commonly encountered in toxicologic pathology. In the first mechanism, the chemical may bind to the receptor and increase its activity, leading to tumor formation. In the second mechanism, the chemical may interfere with the downstream effects of a receptor such that feedback mechanisms result in increased activation of a receptor by endogenous ligand, resulting in tumor formation.

Many receptors have been incriminated in carcinogenesis. For example, the carcinogenic properties of various synthetic and naturally occurring compounds have been hypothesized to cause cancer by estrogen receptor activation. The estrogen receptor poses an interesting challenge, since some chemicals can be estrogenic or antiestrogenic, depending on the cell- and tissue-specific context under evaluation. Further complexity results from the ability of certain estrogenic compounds to be metabolized to yield promutagenic metabolites in a tissue-specific fashion, suggesting that the same chemical can elicit genotoxic and nongenotoxic responses.

In some instances, chemical carcinogens induce phenotypic changes that are reminiscent of receptor-mediated responses, even though a specific receptor has not been identified. For example, phenobarbital causes increases in smooth endoplasmic reticulum and induction of mixed-function oxidases such as CYP2B1/2. Prolonged administration of phenobarbital and related compounds results in mouse liver cancer. The mechanism linking treatment to phenotypic changes is unknown, but similarity of response suggests the presence of a phenobarbital receptor, and some preliminary evidence for the existence of a phenobarbital modulated transcription factor has recently been presented. This receptor could also be central to the mechanism of liver carcinogenesis.

Other identified mechanisms of receptor-mediated carcinogenesis involve altered feedback regulation, leading to increase in trophic hormones that result in increased stimulation of certain tissues. Examples exist for thyroid follicular cells, pancreatic acinar cells, and gastric enterochromaffin-like cells. In the case of thyroid follicular cells, administration of chemicals that block production or increase

elimination of thyroid hormones leads to sustained pituitary TSH stimulation and eventually hyperplasia and neoplasia in rats.

Peroxisome proliferators

Peroxisome proliferators comprise a class of chemicals that similarly cause hepatic adaptation characterized by liver enlargement, hepatocellular hyperplasia, and increases in the peroxisomal compartment of hepatocytes. Interest in peroxisome proliferators stems from the varied and often extensive commercial applications associated with some of the chemicals, the potential for human exposure, and the striking association between the hepatic adaptation and eventual development of liver cancer following long-term exposure in rats and mice. Concern over peroxisome proliferators as a class of chemicals has centered on clarifying the potential mechanism that may explain their carcinogenic activity in rodent liver. Identification of any chemical as a peroxisome proliferator does not preclude its having other toxicologic or carcinogenic properties. However, the consistent association of peroxisome proliferation and induction of cancer in the livers of both rats and mice has become the overwhelming basic issue to be resolved for this class of chemicals.

Peroxisome proliferators have quite varied chemical structure, but most have or can be metabolized to intermediates that have an acidic functional group. Since interaction with genomic DNA has been ruled out for most, if not all, peroxisome proliferators, they are considered to lack genotoxic activity. Peroxisome proliferators are particularly well represented among hypolipidemic fibrate drugs such as gemfibrozil and clofibrate. Peroxisome proliferators have also been identified among other drug classes such as leukotriene antagonists. In addition to use as drugs, other peroxisome proliferators are marketed as crop protectants (e.g., lactofen and fomesafen). Finally, certain of the phthalate ester plasticizers, notably di-(2-ethylhexyl)phthalate (DEHP), are peroxisome proliferators. Thus the range of human exposure to peroxisome proliferators can extend from extremely low environmental exposures in the case of phthalates and crop protectants to pharmacologically active exposures in the case of drugs.

Despite the concern over rodent hepatocarcinogenicity as an indication of an increase in risk of human cancer, experience and limited epidemiological studies

have failed to detect any increased cancer in humans following chronic exposure to fibrate drugs. Since slight increases in cancer in the human population at risk would be difficult to detect, additional information on the mechanism of rodent hepatocarcinogenicity has become crucial to addressing this concern.

A major advance in identifying the mechanism of response to peroxisome proliferators has been the discovery of the peroxisome proliferator-activated receptor-alpha (PPARα). This receptor is a member of the steroid hormone receptor superfamily. It is characterized by a zinc finger DNA binding domain and an activation domain. Activation of PPARα involves heterodimerization with another superfamily member, RXRα, and binding to a direct repeat-1 response element (peroxisome proliferator response element, PPRE) in the promoter-enhancer region of certain genes. In this way, PPARα mediates the transcription of genes that characterize the response of liver to peroxisome proliferators. In cultured cells, most peroxisome proliferators or their metabolites can activate PPARα to induce transcription of reporter genes containing the PPRE.
Endogenous activators of PPARα include certain unsaturated fatty acids and eicosanoids. In some instances, direct binding of PPARα activators to the receptor has been demonstrated.

The role of PPARα in mediating the response of liver to peroxisome proliferators has recently been elucidated using PPARα knockout mice. The increases in transcription of several genes for peroxisomal fatty acid oxidation enzymes and the increase in the peroxisomal volume compartment seen in wild-type mice following administration of certain peroxisome proliferators were abolished in PPARα knockout mice. The hyperplasia and hepatomegaly induced by peroxisome proliferators were also abolished in PPARα knockout mice. Evidence from a long-term study with one peroxisome proliferator, the experimental hypolipidemic drug WY-14,643, indicates that PPARα knockout mice are also resistant to liver carcinogenesis by peroxisome proliferators. Therefore studies with knockout mice suggest that all liver responses caused by peroxisome proliferators depend on PPARα. Interestingly, the hypolipidemic effects of fibrate drugs are also attenuated in the knockout mice.

Although all the liver responses caused by peroxisome proliferators appear to be mediated by PPARα, the receptor knockout mice studies did not help to clarify

which receptor-mediated responses in the liver are responsible for the development of cancer. In one hypothesis concerning the mechanism of carcinogenesis by peroxisome proliferators, an indirect mechanism of DNA damage has been proposed to occur in livers of rodents. This oxidative damage is theorized to result as a consequence of increased $H_2O_2$ production by peroxisomes. Consistent with this mechanism is the rather substantial increase in the mRNA and activity of fatty acyl-CoA oxidase, the rate-limiting enzyme of the peroxisomal β-oxidation pathway, following sustained exposure to a peroxisome proliferator. In the peroxisome, this oxidase activity produces $H_2O_2$ that could escape to damage DNA, presumably via production of OH· radical or other oxygen radical intermediates formed proximal to nuclear DNA. Increases in levels of a marker of oxidative damage, 8-hydroxydeoxyguanosine (8-OHdG), in liver DNA following long-term exposure to peroxisome proliferators have been observed in support of this mechanism of DNA damage.

To clarify the role of peroxisomal acyl-CoA oxidase in liver carcinogenesis, an acyl-CoA oxidase knockout mouse strain was established. Interestingly, these mice develop spontaneous increases in the peroxisomal compartment of the hepatocytes and an elevated susceptibility to spontaneous liver cancer. Taken together, these observations suggest that buildup of substrates for acyl-CoA oxidase can lead to activation of PPARα, which, in turn, results in liver cancer, albeit through an acyl-CoA oxidase independent mechanism. This indicates that other PPARα-mediated responses are critical to the development of cancer in rodent liver.

The ability of peroxisome proliferators to increase hepatocellular replication represents an attractive mechanism to explain their carcinogenic activity. The level of hyperplasia observed early in the course of sustained exposure is similar to peroxisomal enzyme induction in that it is not an consistent quantitative predictor of eventual carcinogenic activity. However, it does establish that activators of PPARα can lead to cell proliferation in hepatocytes. During the pathogenesis of peroxisome proliferator-induced liver cancer, the appearance of distinct focal proliferative lesions composed of basophilic hepatocytes precedes and resembles the cancers that subsequently arise. The sustained cell proliferation in these lesions is crucial to the eventual development of cancer, in that cessation of administration of the peroxisome proliferator leads to diminished cell proliferation and lesion regression. Which of the genes modulated by PPARα are responsible for cell proliferation in the

reversible focal proliferative lesions is not yet clear. However, identification of promising candidate genes suggests a strategy for knockout mouse constructs that would be resistant to the carcinogenicity of peroxisome proliferators, even if expression of functional PPARα is intact.

The emergence of PPARα as a mediator of carcinogenesis by peroxisome proliferators has profound implications for the assessment of human cancer risk. Species differences in sensitivity to peroxisome proliferators have been characterized in a variety of in-vitro and biopsy studies. Some generalizations can be made from these studies. In-vitro studies have concentrated on the induction of acyl-CoA oxidase or other enzymes, and human hepatocytes are much less sensitive or completely refractory to peroxisome proliferators as compared with hepatocytes from rats. Biopsy studies of patients receiving fibrate therapy have failed to detect changes in the peroxisome compartment that are readily detected in rats and mice. The basis of the refractory nature of human hepatocytes to peroxisome proliferators is currently under study. Although humans appear to express functional PPARα, levels of PPARα expression in human liver are much lower than in rats and mice.

The extent to which PPARα-mediated responses can increase the risk of cancer in rodents, along with the refractory nature of human liver to these responses, presently suggests that a straightforward extrapolation of cancer risk between species is not appropriate. The low potency of most chemicals and low level of exposure in the nonclinical setting, coupled with the species differences in response, may indicate that few, if any, humans would have increased risk of cancer as a result of typical exposure to industrial and agricultural peroxisome proliferators. In the clinical setting where pharmacological responses are being elicited by peroxisome proliferators, assessment of cancer risk should depend upon the likelihood of transcriptional activation by PPARα or other PPAR subtypes in liver or other responsive tissues.

## Conclusions

Receptor-mediated carcinogenesis has emerged as an important mode of action to be considered in clarifying the assessment of chemical risks. Recent studies of peroxisome proliferator carcinogenesis indicate that the receptor PPARα is

central to the mechanism of liver carcinogenesis for a variety of chemicals. These findings illustrate the utility of knockout mice in characterizing the potential risk of other chemicals that cause cancer via specific receptor activation.