Regulation of the Multidrug Resistance Genes by Stress Signals

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Abbreviations:
HSF,Heat Shock Factor; IFN,Interferon; IL-1β, Interleukin-1β; IL-6, Interleukin-6; LIF, Leukemia Inhibitory Factor; *mdr*1,General term for human *MDR1* and rodent *mdr1a*, *mdr1b* multidrug resistance genes; *MDR1, MDR3,*Human multidrug resistance genes; *mdr1a, mdr1b, mdr2,*Murine and rat multidrug resistance genes; MRP, Multidrug resistance associated protein; *mrp,* Multidrug resistance associated protein genes; PKA,Protein Kinase A; PGP, P-Glycoprotein; TGF, Transforming Growth Factor; TNF,Tumor Necrosis Factor

ABSTRACT

Transporters in the body play a large role in the distribution and elimination of many clinically important therapeutic substances. Of these, perhaps the one that has been best studied is P-Glycoprotein (PGP), a 170 kDa membrane-bound protein which has been implicated as a primary cause of multidrug-resistance in tumors. An understanding of the physiological regulation of these transporters is key to designing strategies for the improvement of therapeutic efficacy of drugs which are their substrates. To that end, we examine herein the current state of understanding of the molecular regulation of PGP by a variety of endogenous and environmental stimuli which evoke stress responses including cytotoxic agents, heat shock, irradiation, genotoxic stress, inflammation, inflammatory mediators, cytokines and growth factors.

INTRODUCTION

Transporters in the body play a large role in the distribution and elimination of many clinically important therapeutic agents. P-Glycoprotein (PGP) is a 170 kDa ATP-dependent membrane-bound transporter that is known to confer resistance to a variety of structurally unrelated, clinically important antineoplastic agents (1-3). This phenomenon is generally known as multidrug resistance. PGP is encoded by the *mdr*1 genes which includes *MDR1* in humans and *mdr1a and mdr1b* in rodents. Overexpression of the *mdr*1 gene products has been implicated as a primary mechanism of tumor drug resistance (4-6), particularly in tumors arising from tissues which normally express PGP (e.g., the liver, kidney, intestine and blood-brain barrier). Although their physiological function is not clearly defined, the *mdr*1 gene products are thought to play a role in the protection of organisms against toxic xenobiotics. Studies in *mdr1a* (-/-) knockout mice have demonstrated increased sensitivity to as well as increased concentrations of xenobiotics such as invermectin, vinblastine, cyclosporin A and digoxin (7,8). Indeed, twenty to fifty fold higher brain concentrations of cyclosporin A and digoxin are found in *mdr1a* (-/-) knockout mice (9). Gene products of other multidrug resistance gene family members do not play a role in the drug resistant phenotype (10). These include human *MDR3* (*mdr2* in rodents), which encodes for a phospholipid transporter and *SPGP* (the sister gene of PGP), which encodes for a hepatic bile salt transporter.

While antineoplastic agents are important substrates of PGP, a variety of other clinically relevant drugs are also transported by PGP. Therefore, in addition to examining PGP overexpression in tumor cells, understanding physiological mechanisms of PGP regulation may help us to explain subject variability in drug disposition. Identification of gene sequences and recent advances in molecular biology have resulted in an explosion of knowledge regarding the genetic regulation of PGP. Thus delineating these regulatory pathways may enable us to predict and manipulate expression of the *mdr*1 genes in order to improve the clinical effectiveness of PGP substrates.

As PGP functions to protect cells from harmful chemicals and metabolites, it is plausible that these transporters play an important role in the cellular response against stress. Furthermore, numerous “stress-evoking” stimuli have been reported to alter *mdr*1 gene expression. The promoter regions and upstream regulatory sequences of the human, mouse, hamster and rat *mdr*1 genes have been identified and characterized (11-14). A summary of what is currently known about the regulatory sequences

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of the *mdr*1 genes in human, mouse and rat is presented in Figures 1-3 (compiled from references 15-20). In each of the *mdr*1 gene sequences, putative binding sites for various "stress" transcription factors are found, including those for AP-1, Sp-1, AP-2, NF-Y, and C/EBPβ (also known as NF-IL6). While the functional significance of these binding sites have yet to be established, it is likely that these sites and transcription factors are the targets of environmental and pathological signaling pathways which evoke the stress response. This review will primarily focus on what is currently known about the effects of heat shock, irradiation, genotoxic stress, inflammation and inflammatory mediators on *mdr*1 expression and regulation.

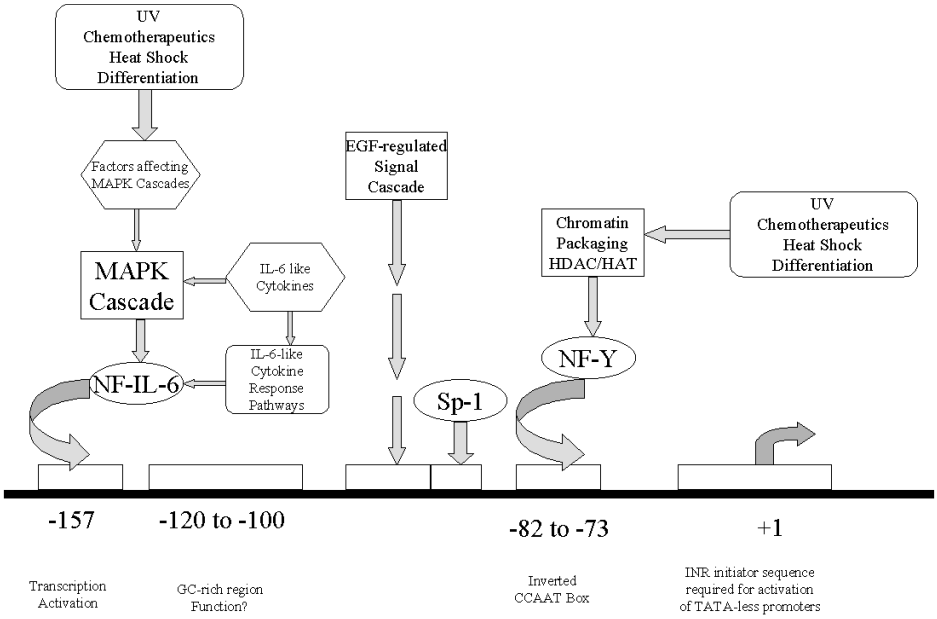

Figure 1: A schematic of the human *MDR1* promoter region, showing the relative locations of transcription factor binding sites, as well as interacting transcription factors and signal transduction pathways.

Mechanisms of mdr1 gene regulation

Although expression of PGP has been frequently examined, regulatory mechanisms of this gene product are complex and still poorly understood. Evidence from numerous studies indicate that expression and activity of PGP can be controlled either pre- or post-transcriptionally by a myriad of environmental influences. For instance, protein kinase C activators which increase PGP activity and drug resistance have been found to enhance *mdr*1 gene expression *via* both transcriptional and translational pathways (21). Modulations in protein stability, plasma membrane incorporation, mRNA stability and processing, gene transcription and gene amplification have each been reported for PGP (1-5, 13). Of these, alterations in PGP expression that occur at the level of mRNA are perhaps the most frequently observed (22). Although increased mRNA levels generally occur as a result of enhanced gene transcription rates, prolonged cellular exposure to several cytotoxic drugs have also been reported to induce *mdr*1 gene overexpression through both gene amplification (4,5) as well as by increased mRNA stability (23). Moreover, *mdr1b* overexpression in primary cultures of rat hepatocytes occurs primarily due to an increased mRNA stability (24,25). As it is thought that changes in mRNA stability may be tied to cell integrity, it is possible that observed decreases in *mdr1b* mRNA degradation in cultured cells could result from cellular stress and tissue disruption imposed by collagenase treatments. Furthermore, cytoskeletal disruption of rat hepatocytes by cytochalasin D prevents changes in *mdr1b* mRNA stability upon culturing (25).

Heat shock

Heat shock proteins are proteins that are synthesized in response to stressful environmental stimuli such as heat. These often include proteins that are thought to help in stabilizing and repairing cell damage. It is likely that efflux transport proteins such as PGP, which are involved in the removal of toxic metabolites and by-products, play an active role in this protective mechanism. Identification of two strong heat shock consensus elements within the human *MDR1* gene promoter, as well as an observed *in vitro* increase in *MDR1* mRNA following cellular exposure to high temperature and toxic heavy metals suggest that *MDR1* could function as a heat shock gene. It has been shown that basal activity of the *MDR1* promoter requires heat shock factor (HSF-) mediated transactivation (26). Indeed, inhibition of the DNA-protein complex formation between HSF and its response element has been found to block *MDR1* basal transcription, sensitizing drug resistant cells to anticancer drugs (27). Furthermore, inhibition of protein kinase

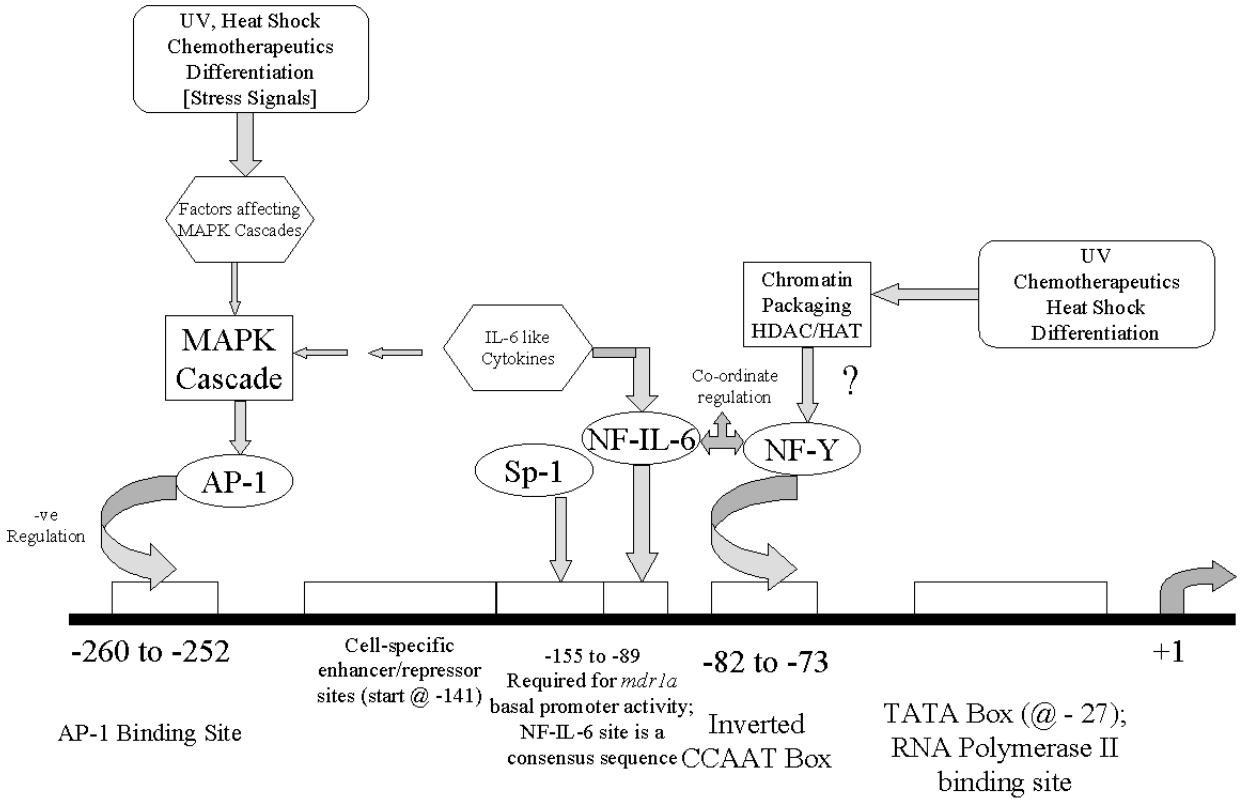

Figure 2: A schematic of the murine *mdr1a* and *mdr1b* promoter regions, showing the relative locations of transcription factor binding sites, as well as interacting transcription factors and signal transduction pathways. The information presented here is an amalgam of what is known for both *mdr1a* and *mdr1b*.

A (PKA) suppresses HSF DNA-binding activity as well as reducing expression of the heat shock proteins hsp90 and hsp70 (26). Cells treated with antisense oligonucleotides to both hsp90 and *MDR1* have been demonstrated to display vastly decreased PGP half-lives and increased doxorubicin sensitivity to that observed in controls or to that of cells treated with the antisense oligonucleotide to *MDR1* alone (28). In these studies hsp90, which could be both co-precipitated as well as co-induced with PGP, was implicated as a possible "chaperone protein" for PGP and is thought to somehow aid in the maintenance of PGP functional activity and protein half-life. Thus suppression of hsp90 expression would likely result in decreased PGP half-life and activity. On the other hand, further experiments conducted by Kim *et al* (29) have shown that the heat shock element may be involved in alterations of *MDR1* transcription rates through pathways that are depen-

dent upon PKA and the raf oncogene. That is, raf activation by heat shock or sodium arsenite, which stimulates the heat shock response, resulted in an induction of PGP activity whereas inhibition of PKA activity using 8-Cl-cAMP, blocked the heat shock potentiation of PGP activity (29). Subsequent studies have established that protein kinase inhibition with 8-Cl-cAMP results in a reduction in *MDR1* gene transcription rates (30). Taken together, these data indicate multiple pathways of control of *MDR1* expression by cellular pathways that define the heat shock response.

Cells can be made resistant to heat shock (a phenomenon known as "thermotolerance") in the same manner as they can be made drug resistant: Constant and increasing exposure to thermal stresses followed by drug selection conditions. Indeed, in rat hepatoma cells treated in such a manner, there is a correlation between the induction of HSF and the induction of *mdr*1 gene expression and activity (31). However, *in vivo* studies contradict these findings, as Vollrath *et al* (32) presented evidence showing that the rat *mdr1* genes are not induced during heat shock. Nevertheless, as a substantial body of evidence exists detailing modulation of *mdr*1 expression by heat shock, the discrepancies in these studies may be due to difficulties incurred in controlling a heat shock response in a whole animal. Differences in regulation between normal and hepatoma cells, and between species (human and rat) may also play a role. However to date, it seems clear that *MDR1* participates as a heat shock response gene in humans. More information in this area may evolve as it is now thought that the heat-shock proteins may play an undefined role in cancer and in the development of drug resistance.

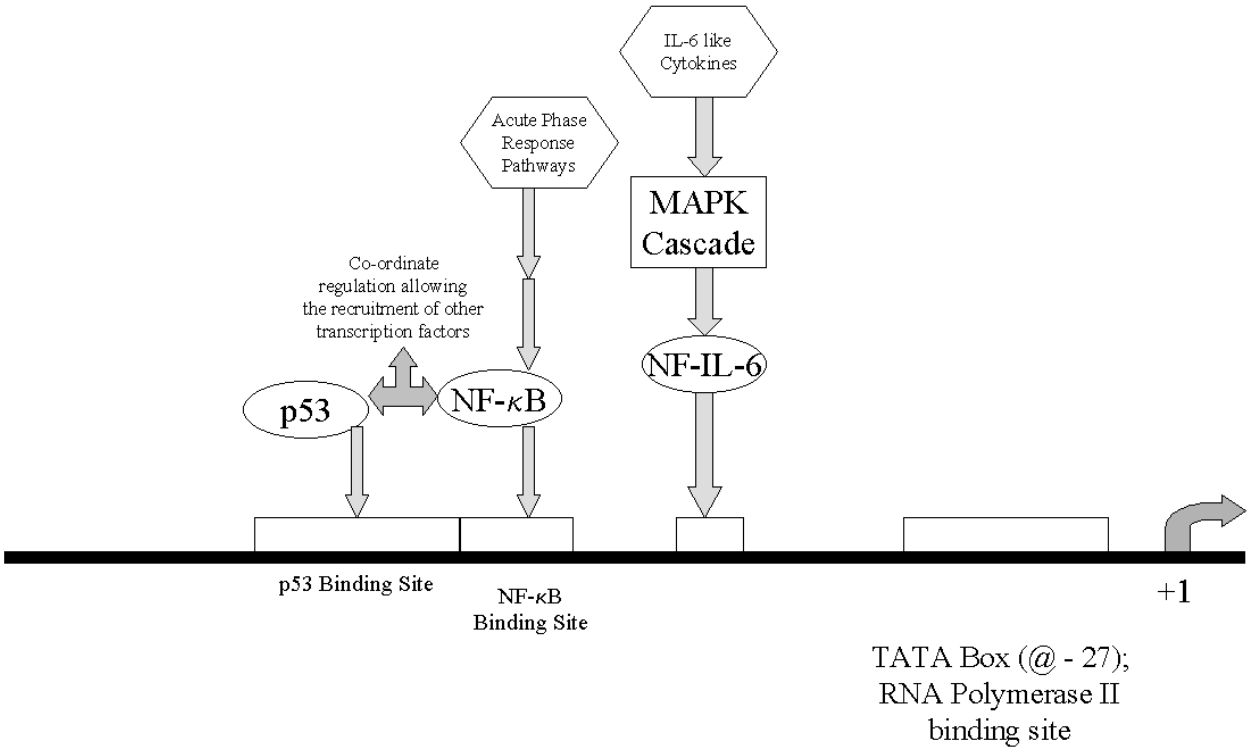

Figure 3: A schematic of the rat *mdr1b* promoter region, showing the relative locations of transcription factor binding sites, as well as interacting transcription factors and signal transduction pathways. The majority of rat *mdr1a* promoter regions have yet to be sequenced

Irradiation

In addition to initiating genetic mutations, ionizing radiation may initiate cellular responses that can ultimately affect *mdr*1 gene expression. Generally, irradiation has been observed to evoke increased rather than decreased *mdr*1 expression. It has been demonstrated

that induction of *MDR1* expression incurred by ultraviolet irradiation results from increased *MDR1* gene transcription rates (33,34). Signaling of this induction is thought to primarily occur through a CCAAT box/NF-Y-binding site that is found in the *MDR1* promoter (shown in Figure 1). Fractionated X-irradiation has also been found to increase PGP expression in CHO cells due to an increased protein stability and half-life with corresponding decreases in turnover rates (35-38). Indeed, increased PGP half-lives of more than 40 hours are found in irradiated cells as opposed to 17 hours in the control cell populations (36,37). Furthermore, the enhanced stability of PGP reportedly occurs without concomitant increases in mRNA levels (35,38).

Evidence also suggests that the superstructure of chromatin plays a role in transcriptional regulation during UV irradiation. Specifically, the histone acetyltransferases and deacetylases that modulate DNA packaging into histones are believed to be involved. Jin and Scotto (39) reported that incubation of a human carcinoma cell line (SW620) with an inhibitor of histone deacetylase induces a 20-fold increase in *MDR1* mRNA levels (39). The authors report that this induction likely occurs through an increased transcription, requiring the sequence from –82 to –73, which contains an inverted CCAAT box element, as point mutations of this sequence were found to abolish promoter response to the histone deacetylase inhibitor. The authors also postulated that ratios of acetyltransferase to deacetylase activities could be important in *MDR1* regulation, with hyperacetylation leading to gene activation. Similarly, gel mobility shift assays establishing binding of NF-Y to the inverted CCAAT box and the involvement of NF-Y in intrinsic histone acetyltransferase activity also appear to indicate regulatory mechanisms of human *MDR1* gene expression *via* chromatin acetylation/deacetylation pathways (39). It is unclear whether *MDR1* overexpression during radiation exposure is a part of the cellular response to being irradiated or a side effect due to the stimulus. As discussed previously, sequences containing inverted CCAAT boxes were implicated by Ohga *et al.* (33,34) and by Jin & Scotto (39) in *MDR1* induction imposed by UV irradiation. Of note is that this promoter sequence has also been implicated in induction imposed by various stimuli including differentiation (40), heat shock (41) and cytotoxic drugs (33) as this sequence is thought to play a role in maintaining basal *MDR1* promoter activity (41,42). This implies that *MDR1* may be induced by radiation through a general non-specific cellular response to environmental stress. Nevertheless, as radiation therapy is frequently used concurrent with antineoplastic drugs in cancer treatment, the effect of irradiation on cellular gene expression is certain to receive more attention in the years to come.

Genotoxic stress

Recently, much progress has been made in defining the signal transduction pathways that mediate cellular responses to DNA damage. Contributing to this response are multiple pathways involving alterations of phosphorylation of proteins and transcription factors which occur through several distinct protein kinases (ERK, JNK/SAPK, and p38/HOG1) as well as the tumor suppressor protein p53. In particular, the cyclic AMP responsive transcription factors such as NF- 6B, AP-1, Sp1 and CREB transduce signals in response to protein kinase C activation. To date, several lines of evidence demonstrating a correlation between protein kinase activity and *mdr*1 expression suggest that activation of cyclic AMP-dependent protein kinases may be involved in induction of the multidrug resistant phenotype in tumor cells (43).

The c-Jun NH_2-terminal protein kinase (JNK), a member of a well-characterized mitogen activating protein kinase cascade (44-47) is activated in response to many stressful stimuli including growth factors, phorbol esters, heat shock, UV irradiation, protein synthesis inhibitors, and inflammatory cytokines (46-51). It has been reported that JNK is activated in human carcinoma cells by treatment with a number of different anticancer drugs and this activation of JNK correlates with increased *MDR1* expression (44). It is thus believed that JNK may play a role in cellular development of the multidrug resistant phenotype. JNK is known to phosphorylate and activate c-jun, which comprises half of the heterodimeric AP-1 transcription factor (51). It is known now that there are AP-1 binding sites on the promoters of *mdr*1 genes across species (52) and a positive correlation between AP-1 activation and *mdr*1 transcription has been reported (53). It is therefore possible that induction of *mdr*1 expression correlating with JNK activation could be traced to trans-activation by AP-1. Other agents which activate

the JNK or protein kinase cascades may also affect *mdr*1 expression in this manner.

DNA damage is perhaps the best-studied stress that activates p53, and recent data implicate phosphorylation at N-terminal serine residues as critical in this process. It is interesting to note that many members of the mitogen activating protein kinase cascades, because of their roles in controlling cellular growth and proliferation, are oncogenes (c-H-raf and c-H-ras, for example). It has been shown that transformation of rat liver cells with v-H-ras or v-raf oncogenes causes an induction of *mdr1*/ PGP expression (54). In addition to being under the indirect control of those oncogenes, *mdr1b* in rat hepatoma cells is thought to function as a p53 response gene. Indeed, it has been demonstrated that presence of the p53 response element and the adjacent NF-κB binding site in the *mdr1b* promoter are both required for basal promoter activity (55,56).

The inflammatory response and cytokines

Induction of an acute inflammatory response in experimental models of inflammation in rats (57) and mice (58) has been demonstrated to decrease the hepatic expression and activity of PGP at the level of mRNA. Findings indicating a down-regulation of the *mdr1a*, *mdr1b* and *mdr2* genes were obtained in both species with two different inflammation models. These included the turpentine model, which produces a localized inflammatory reaction and the bacterial lipopolysaccharide model, which produces a systemic endotoxemia. Furthermore, experiments in primary hepatocyte cultures treated with lipopolysaccharide also show a reduction in PGP expression and activity (unpublished data). On the other hand, somewhat different results were found in the livers of endotoxemic rat livers by Vos *et al* (59). His results indicated up-regulation of *mdr1b*, down-regulation of the bile salt transporter *spgp* while levels of *mdr1a* and *mdr2* remained unchanged. Although neither quantitative nor statistical analysis of results were presented (59), it is likely that the degree of endotoxemia in the rats may play a role as this may alter the pattern and extent of cytokine release. Indeed, we found that adjuvant-induced arthritis in rats, which is a classical animal model of chronic inflammation, did not significantly alter the hepatic expression of PGP (unpublished data). Furthermore, other studies in the laboratory indicated that renal and intestinal expression of the *mdr1* genes remain unchanged in LPS or turpentine-injected rats, suggesting that suppression of the *mdr* genes during acute inflammation is both a complex and liver-specific phenomenon.

It is well known that the majority of effects seen during an acute inflammatory response are associated with the release of a few of the pro-inflammatory cytokines. In particular, it has been demonstrated that interleukin 1β (IL-1β) and IL-6, and to a lesser extent, tumor necrosis factor (TNF-) α, are the principle mediators involved in controlling the hepatic gene expression of numerous glycoproteins, as well as the cytochrome P450 drug metabolizing enzymes during inflammation. Therefore, it is likely that these mediators are also involved in PGP regulation and the control of *mdr* gene expression during an inflammatory response. Indeed, *in vitro* treatments of cultured hepatocytes with recombinant IL-1β and IL-6 elicit dose- and time-dependent reductions in PGP expression and activity (60-62). Results demonstrating decreases in *mdr*1 mRNA in IL-6 but not IL-1 treated cells (60-62), suggests that IL-1β-mediates effects on PGP expression *via* post-translational mechanisms, whereas IL-6 likely influences PGP expression by either decreasing *mdr*1 mRNA stability or reduced transcription rates. Experiments are underway to clarify this matter. *In vivo* experiments in mice have also demonstrated similar cytokine-mediated effects on PGP and *mdr*1 expression (58). These data also support the hypothesis that IL-6 is primarily responsible for down-regulation of PGP expression and activity during an acute inflammatory response.

Several studies also indicate that TNF-α, which primarily acts through NF-κB, suppresses *mdr1b* gene expression. These *in vitro* studies report down-regulation of PGP protein and *MDR1* gene expression as well as enhanced chemosensitivity in continuous human intestinal cell lines treated with TNF-∀ (63-65). A binding site for NF-κB exists on the *mdr1b* promoter (55) which may implicate the potential involvement of this transcription factor in *mdr1b* down-regulation. On the other hand, others have also observed a TNF-α mediated induction of *mdr1b* expression in cultured rat hepatocytes that can be suppressed by addition of the anti-inflammatory agent dexamethasone (66,67). The apparent discrepancies in these studies are likely due to species and TNF treatment differences. Furthermore, as different cell

types are unique in their ability to produce and release other cytokines, the use of intestinal or hepatic cells in these studies likely influence cellular exposure to other cytokines which could contribute to their dissimilar findings.

In terms of species differences, although we have observed that the inflammatory response mediates a suppression of PGP in rats (57) and mice (58), this phenomenon has yet to be examined in humans. Several reports indicate a diminished *MDR1* gene expression and/or potentiation of chemosensitivity in human colon carcinoma cell lines incubated with a number of these cytokines including IFN-γ, TNF-α, IL-2 and leukoregulin (63-65,68-70). While information in this area is limited, IFN-γ, TNF-α effects are mediated through an inhibition of *MDR1* gene transcription (63,64). Studies with IFN-α have also demonstrated an IFN-α mediated downregulation of *MDR1* in a human hepatoma cell line (71). Other cell types have not produced consistent results with cytokine treatments (72). *In vivo* studies have not yet been conducted however, observations of therapeutic synergism have been reported in patients given combinations of cytotoxic drugs with IFN or TNF (73,74).

While the molecular pathways involved in cytokine-mediated regulation of *mdr*1 gene expression have not been delineated it is likely that the down-regulation of *mdr*1/PGP in hepatocytes occurs through inhibition of *mdr*1 gene transcription. It is felt that the cytokines mediate their effects through unique signal transduction pathways involving only a handful of nuclear transcription factors (NF-6B, C/EBP and APRF) (75). Changes in hepatic protein production during an inflammatory response are thought to be primarily mediated through the nuclear factor NF-IL6 (also known as C/EBPβ) which belongs to the C/EBP transcription factor family (75,76). During an inflammatory response or after exposure to IL-1, IL-6 or TNF, dramatically increased levels of NF-IL6 function as both a positive and negative regulator of transcription within the liver (76). Binding sites for NF-IL6 and other C/EBP transcription factors have been identified on the promoter region of the *mdr*1 genes (15,16,19). Thus transcription control through members of the C/EBP family such as NF-IL6 may provide a possible cellular signaling pathway by which inflammation and inflammatory mediators suppress PGP expression (20).

Preliminary investigations in our laboratory with Leukemia Inhibitory Factor (LIF) also indicate a complex pattern of *mdr*1 mRNA suppression (77). It is known that IL-6 and LIF activities are mediated through similar pathways: MAPK activation of NF-IL-6 (C/EBPβ) and/or induction of the acute phase response factor through the JAK/STAT pathway (78). However, the mechanisms through which both cytokines act on PGP expression have not yet been elucidated. The anti-proliferative cytokine, transforming growth factor (TGF-) β_1, which is released during an acute inflammatory reaction also appears to influence *mdr*1 gene expression in a complex manner. Long-term exposure to TGF-β_1 induces drug resistance by induction of *MDR1* mRNA expression (79) whereas short-term exposure to TGF-β_1 in glioblastoma cells has been reported to decrease *MDR1* expression (80). Initial studies in cultured rat hepatocytes in our laboratory also demonstrated an induction of *mdr1b* mRNA after 24 hours of exposure to TGF-β_1 (77). It is known that TGF-β_1 operates through a family of transcription factors and associated proteins known as the Smads, which interact with AP-1 (81-85). As AP-1 binding sites exist in the promoters of members of the *mdr*1 gene family, this may provide a link to TGF-β_1 modulated effects on PGP/*mdr*1 transcription.

In addition, other growth factors are known to induce and modulate PGP/*mdr*1 expression. In particular, epidermal growth factor and insulin-like growth factor-1 are both known to induce PGP and *mdr1b* expression in a time-dependent manner in cultured rat hepatocytes (86). Aside from likely effects on gene transcription, resulting in increased mRNA levels, it is possible that epidermal growth factor could also have an effect on the post-translational regulation of PGP, *via* phospholipase C and increased phosphorylation of PGP (87). In the case of insulin-like growth factor-1, it is thought that it may induce *mdr*1 gene expression *via* a c-H-ras dependent MAPK signaling cascade (88).

To date, the effects of cytokines on PGP expression have not been fully delineated. In addition to possessing complex patterns of induction and inhibition, the cytokines have many overlapping and synergistic effects. Thus it is likely that the cytokines also interact with *mdr*1 gene expression in a elaborate manner resulting in unique effects dependent upon cytokine concentrations, cell type and species. As many disease

states are associated with changes in cytokine secretion there is a need to explore PGP regulation in both health and disease. Further investigation is also necessary, for although cytokine-mediated mechanisms of *MDR1* down-regulation have promising usefulness in cancer treatments, available information in this area is limited with many discrepancies reported.

The Multidrug Resistance-Associated Protein (MRP)

The overexpression of other drug efflux transporters such as the multidrug resistance-associated proteins (MRP) is beginning to be recognized as playing an important role in the development of drug resistance in tumors. Much in this area needs to be explored. As several members of the MRP transporter family are also induced by cytotoxic drugs (89-92), the potential for environmental and physiological regulation of these genes also exists. Indeed, this field is still in its infancy and promoter sequences of the *mrp* genes have not yet been reported. Thus little is known about the signaling pathways that regulate MRP. However, several pathways of MRP gene regulation appear to occur through stimulation by environmental factors. For example, fractionated gamma-irradiation of a human T-cell leukemic cell line reportedly causes a six-fold increase in levels of *mrp1* mRNA levels (93, 94). It therefore seems likely that *mrp1* is somehow involved in the immediate cellular response to radiation. As *mdr*1 levels are also increased through irradiation (34,94), induction and overexpression of *mdr*1 and *mrp1* following radiation therapy likely contribute to the clinical development of resistance in tumors. Furthermore, numerous mechanistic links between p53 activity and *mrp1* and *mdr*1 expression have also been reported in drug resistant cells. Wild type p53 reportedly suppresses *mrp1* transcription (95) and significant increases in *mrp1* mRNA levels are seen in p53 inactivated or mutated cell lines (96). This negative regulatory effect of p53 is thought to occur through effects on the transcription activator Sp-1 (95). Likewise, levels of *mdr1a* mRNA and PGP are also markedly elevated in p53 inactivated rodent hepatoma cells (97) whereas rat *mdr1b* gene expression and basal *mdr1b* promoter activity requires and is induced by wild type p53 activity (56). Conversely, a recent study, indicating a lack of effect of heat shock on *mrp1* expression in esophageal cancer cells, may suggest that *mrp1* does not function as a heat shock gene (98). However, this evidence is limited and further studies in alternate cell lines are necessary to fully establish whether MRP induction contributes to drug resistance elicited by heat shock treatments.

Interestingly, we as well as others (89, 102) have seen that induction of an acute inflammatory reaction reduces the expression and activity of *mrp2* at the level of mRNA. This downregulation of *mrp2* likely occurs due to pro-inflammatory cytokine release, as our preliminary studies have observed reductions in *mrp2* mRNA and protein expression in mice treated with IL-6, IL-1β or TNF-α (unpublished results). Furthermore, the anti-inflammatory agent, dexamethasone has been shown to prevent inflammation-induced changes in *mrp2* expression (99). It appears that other MRP family members may also be regulated through cytokine-mediated pathways as *mrp1* mRNA expression is reportedly diminished in human hepatoma cell lines upon interferon and TNF-α treatment (100,101). It will be interesting to see how the field develops in a few years, and to compare more fully the regulation patterns of the *mdr* and *mrp* gene families.

Summary

A number of environmental stimuli are known to affect the expression of the *mdr*1 genes. In humans, it has been suggested that *MDR1* may function as a heat shock gene and its expression may be modulated by the numerous agents which trigger these signaling pathways. Ionizing radiation has also been shown to up-regulate *mdr*1 gene expression across species *via* a number of mechanisms. Evidence demonstrates UV-irradiation modulates human *MDR1* gene transcription through chromatin packaging mechanisms; a novel regulatory pathway first seen with the *MDR1* gene. Other stress stimuli including a variety of cytokines and growth factors have also been demonstrated to elicit effects on *mdr*1 gene expression. Although much has been reported on *mdr* gene expression, the mechanisms involved in its regulation are still relatively unknown. It is likely that a limited number of transcription factors possessing binding sites on the *mdr*1 promoters are involved in stress-stimulated regulatory pathways. For instance, *mdr1* induction by irradiation appears to be part of a general response system via activation of NF-Y. However, many environmental stress signals also trigger the JNK cascades for AP-1 activa-

tion. As the *mdr1* gene contains response elements for AP-1 on its promoter sequences, it seems likely that gene expression may be regulated by cellular stress through alternate pathways stimulated by NF-Y and AP-1.

Further studies to confirm the involvement of these transcription factors will need to be done before our understanding of the regulation of PGP under conditions of physiological stress is complete. Nevertheless, it seems likely that cellular stress and PGP expression are closely linked as this transporter plays a crucial role in the protection of cells from toxic products released during environmental stress. As PGP expression is an important in the clinical efficacy of drugs in both normal and malignant cells, it is anticipated that knowledge of regulatory mechanisms may aid in identifying and developing novel therapeutic strategies to modulate *mdr*1 gene expression.

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