REVIEW

Multidrug resistance 1 gene (P-glycoprotein 170): an important determinant in gastrointestinal disease?

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The interface between luminal contents and intestinal epithelium constitutes the largest area of interaction between the host and the environment. There is now strong evidence that the gene product of the multidrug resistant pump (MDR) plays a critical role in host-bacterial interactions in the gastrointestinal tract and maintenance of intestinal homeostasis. This review highlights the efflux mechanism in the intestinal epithelium which is mediated by the multidrug resistant pump, also known as P-glycoprotein 170. Current studies promise to provide further insights into the contribution of the MDR1 gene in the pathogenesis of inflammatory and malignant disorders of the gastrointestinal tract.

INTRODUCTION

The multidrug resistance pump (P-glycoprotein) is a 170 kDa phosphorylated and glycosylated plasma membrane protein belonging to the ATP binding cassette superfamily of transport proteins encoded by the multidrug resistance genes (MDR), first described by Juliano and Ling in 1976.¹ In humans, two MDR genes, MDR1 and MDR3 (also called MDR2) have been described; in rodents, three (*mdr1a*, *mdr1b* and *mdr2*).²⁻⁴ Only the MDR1 gene in humans and *mdr1b* and *mdr1a* genes in rodents appear to be involved in drug transport and the development of drug resistance.⁵ The human MDR3 and murine *mdr2* genes encode a P-glycoprotein that does not seem to have a role in drug transport in the intestinal epithelium but has a role in the secretion of phosphatidylcholine into bile in the biliary tract.⁶ In humans, mutations in the MDR3 genes are linked to the development of progressive familial cholestasis.^{7,8}

P-glycoprotein is a transmembrane protein which is 1280 amino acids long and consists of two homologous halves of 610 amino acids joined by a flexible linker consisting of 60 amino acids. Each half has an N terminal hydrophobic domain containing six transmembrane domains followed by a hydrophilic domain containing a nucleotide binding site. The nucleotide binding sites can bind ATP and its analogues and both halves are essential as inactivation of either site inhibits substrate stimulated ATPase activity (figs 1, 2). However, the two sites are likely to be functionally independent and cleavage probably occurs at one site at a time.⁹

In the human gastrointestinal tract, P-glycoprotein is found in high concentrations on the apical surfaces of superficial columnar epithelial cells of the colon and distal small bowel. High levels of P-glycoprotein are also found on the apical surfaces of epithelial cells in small biliary ductules, small ductules of the pancreas, proximal ductules of the kidneys, and adrenal glands.¹⁰ P-glycoprotein is richly expressed on the sub-apical surface of the epithelium of the choroid plexus of the brain (which forms the blood-cerebrospinal fluid barrier) as well as the luminal surface of the endothelium of the blood capillaries of the brain (blood-brain barrier).¹¹⁻¹⁴ In the

SUMMARY

The interface between luminal contents and intestinal epithelium constitutes the largest area of interaction between the host and the environment. Critical to this interface is the ability to allow transcellular permeation of essential luminal molecules such as nutrients, but at the same time to exclude or counter potentially harmful substances in the intestinal lumen. The cells of the mucosal immune system are protected from the large antigenic load in the gut lumen by a single layer of epithelial cells. Studies involving transgenic mice models have highlighted the critical role of the mucosal microflora in the pathogenesis of mucosal inflammation and the importance of the epithelial cell barrier function in providing protection against the extensive stimulation of mucosal immune system by the microflora.

Together with the activity of xenobiotic metabolising enzymes in the epithelium, efflux mechanisms have become the subject of considerable interest in recent studies of gut mucosal defence and pharmacokinetics of drugs. This review concentrates on the efflux mechanism in the intestinal epithelium which is mediated by the multidrug resistant pump (MDR1), also known as P-glycoprotein 170. Recent data strongly implicate MDR1 as a determinant of homeostatic interactions between bacteria and host in humans. Current studies promise to provide further insights into the contribution of the MDR1 gene in the pathogenesis of inflammatory and malignant disorders of the gastrointestinal tract.

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Abbreviations: MDR, multidrug resistance; IBD, inflammatory bowel disease; NFκB, nuclear factor κB; IκB, inhibitory protein κB; SNP, single nucleotide protein; PBL, peripheral blood lymphocytes; CYP3A, cytochrome P450 3A.

Figure 1 Two dimensional hypothetical structure of the P-glycoprotein 170 pump.

haemopoietic system, peripheral blood mononuclear cells, macrophages, natural killer, dendritic cells, and T and B lymphocytes all express P-glycoprotein at varying levels.¹⁵

In the gastrointestinal tract, there is regional variation in P-glycoprotein expression. Various methodologies have been used to investigate this (table 1). Within the small intestine there is clear evidence demonstrating that P-glycoprotein is maximally expressed in the epithelial cells of the ileum with a gradual decrease proximally into the jejunum, duodenum, and stomach. Variations of P-glycoprotein expression across the length of the colon are less well defined.¹⁶

THE ROLE OF P-GLYCOPROTEIN

Host-bacterial interactions

The available literature suggests that P-glycoprotein acts as a transmembrane efflux pump which removes drugs from the cell membrane and cytoplasm. However, the normal physiological function of P-glycoprotein in the healthy gastrointestinal tract remains under investigation and may be complex. Expression of P-glycoprotein on the luminal surfaces of epithelial cells of the small and large intestines, biliary ductules, and proximal tubules of the kidney suggest a role in decreasing¹⁷⁻¹⁸ absorption from the gut¹⁹ and secretion of endogenous and exogenous hydrophobic amphipathic toxins.²⁰

Perhaps the greatest insight into the physiological importance of P-glycoprotein in the gastrointestinal tract has come from the description of the phenotype of genetically engineered mice lacking the *mdr1a* gene. The investigators had been stimulated to develop this model by the finding that the

MDR1 gene is present in a region of the human genome (7q21.1) that may harbour a disease gene involved in susceptibility to inflammatory bowel disease (IBD).²¹ Panwala *et al* demonstrated that spontaneous colitis developed in these *mdr1a* knockout mice when maintained under specific pathogen free conditions.²² This was reversed and prevented by administration of antibiotics, suggesting that the intestinal flora was necessary in the initiation and perpetuation of colitis in this model. Other studies have confirmed that these mice have normal viability, fertility, immunology, and a range of biochemical parameters.¹¹⁻²³ Together with the protective effect of antibiotics, these observations imply that loss of the xenobiotic efflux mechanism underlies the development of colitis in this model. Bone marrow transfer studies involving wild-type donor cells into *mdr1a* deficient recipients demonstrated that colitis develops in these mice because of the *mdr1a* deficiency in the epithelial cells rather than in lymphoid or myeloid cells. Colitis associated with *mdr1a* deficiency has been regarded as a model of ulcerative colitis with the presence of long dysregulated crypts, crypt abscesses, and superficial mucosal inflammation. However, transmural infiltration of B and T cells was also observed, not dissimilar to the histological appearances of Crohn's disease.

Susceptibility to the development of colitis increased as the mice (*mdr1a*^{-/-}) aged.²² The gradual acquisition of commensal luminal organisms in the background of defective efflux capacity of epithelial cells may be the reason, although clearly other effects of aging may be pertinent. Sequential faecal analyses over time are necessary to investigate this hypothesis.

In addition, a recent study has added further intriguing insight into the interaction between P-glycoprotein and the gut flora. In *mdr1a*^{-/-} mice, infection with *Helicobacter bilis* accelerated the development of colitis while *Helicobacter hepaticus* infection delayed the development of spontaneous disease.²⁴ *Helicobacter* induced IBD in *mdr1a*^{-/-} mice may be mediated via a family of bacterial toxins (for example, cytolethal distending toxins) which are virulence factors in certain *Helicobacter* infections. However, it is important to emphasise that these mice developed spontaneous colitis independent of *Helicobacter* infection. These observations suggest that specific luminal bacteria may critically influence the development of colitis and its progression in genetically susceptible animals. Both *Helicobacter bilis* and *hepaticus* have been shown to induce colitis in mice lacking T and B cells (SCID mice).²⁴⁻²⁷

Moreover, these findings are consistent with the hypothesis that *mdr1a* deficient mice developed mucosal inflammation not because of increased permeability but as a result of an increase in bacterial activation of epithelial cells. This effect might be pro or anti-inflammatory and again the identity of

Figure 2 Three dimensional hypothetical structure of P-glycoprotein viewed from above (A) and below (B). NBD, nuclear binding domain; P, pore; TMD, transmembrane domain.

Table 1 Regional variation in P-glycoprotein expression in humans and rodents

Authors	Subjects	Methods	Findings
Yumoto ¹⁹	Mice	Efflux rate via rhodamine-123 assays	High in ileum compared with duodenum
Fojo ¹⁸	Human tissues	MDR1 mRNA	Colon>jejunum>rectum>stomach/oesophagus
Fricker ¹⁷	Human GI tract	MDR1 mRNA	Colon>jejunum/ileum>stomach
Chianale ¹⁶	Rat intestine	mdr3 mRNA and rhodamine-123 assay	Ileum>jejunum>duodenum
Trezise ²⁰	Rat intestine	mdr3 mRNA	Moderate expression in duodenum/jejunum, maximal expression in ileum, and decreased expression from proximal to distal colon

MDR, multidrug resistance; mRNA, messenger mRNA.

the bacterium involved may be the critical determinant. It has been demonstrated that bacterial flagellin signalling through the TLR5 receptor can lead to increased nuclear factor κ B (NF κ B) expression in epithelial cells.²⁸ On the other hand, Neish *et al* demonstrated that certain non-pathological organisms can lead to a block in the ubiquitination of inhibitory protein κ B (I κ Ba) (necessary for I κ Ba degradation) and thus a subsequent block in NF κ B translocation to the nucleus.²⁹ In either of these cases, if these events are caused by bacteria entering the epithelial cells, it is possible to envisage how the function of the epithelial cells can be influenced by a defective efflux transporter and the bacterial contents of the lumen.

Pharmacokinetic effects

The effect of P-glycoprotein on the pharmacokinetics of drugs has been well demonstrated in two studies.^{30–31} In *mdr1a*^{–/–} mice, systemic bioavailability for the substrates digoxin and paclitaxel was markedly increased compared with wild-type mice following oral administration. Similarly, faecal levels of these drugs were reduced following intravenous administration. The range of substrates transported by P-glycoprotein is enormous and includes a variety of structurally and pharmacologically distinct hydrophobic compounds (table 2).

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There is accumulating evidence that interactive alliances also exist between xenobiotic metabolising enzymes and P-glycoprotein. The best defined alliance is between cytochrome P450 3A (CYP3A) and P-glycoprotein. These proteins colocalise in the small intestinal epithelial cells and have a considerable overlap in substrate specificities.³² As P-glycoprotein can influence the intracellular concentrations of many CYP3A substrates, it may also affect the availability of those substrates to CYP3A and thereby the extent of CYP3A metabolism of those substrates.

REGULATION OF P-GLYCOPROTEIN

Regulation of MDR1 gene expression and P-glycoprotein activity is unexpectedly complex and far from being understood. The transcriptional regulators of the human multidrug resistance gene are discussed in detail in the review by Labialle and colleagues.³³ P-glycoprotein dependent drug transport activity depends on the level of expression of the MDR1 gene as well as on the functionality of the MDR1 encoded P-glycoprotein. It is clear that intestinal P-glycoprotein expression and function shows wide interindividual differences, influenced by both environmental and genetic factors.

Pharmacological regulation of P-glycoprotein

Shapiro *et al* showed that P-glycoprotein mediated drug transport was stimulated by the antihypertensive drug prazosin and the hormone progesterone.³⁴ Rifampicin has also been shown to induce P-glycoprotein expression and underlies the

mechanism responsible for reduced digoxin levels during concomitant therapy.³⁵ In healthy male volunteers, the oral bioavailability of digoxin decreased by 30% and intestinal P-glycoprotein levels were induced 3.5-fold during rifampicin therapy. Induction appears to take place at the transcriptional level as MDR1 mRNA levels are also elevated after treatment with inducer drugs.^{36–37} It has been suggested that induction of MDR1 is mediated by the pregnane X receptor (which is also responsible for induction of CYP3A) which binds to PXR response elements situated upstream in the MDR1 gene.³⁸

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Table 2 List of P-glycoprotein substrates

Substrate
Steroid compounds
Aldosterone
Progesterone
Hydrocortisone
Cortisol
Corticosterone
Dexamethasone
Anticancer agents
Doxorubicin
Daunorubicin
Vinca alkaloids
Actinomycin D
Etoposide
Immunosuppressive agents
Cyclosporin
FK 506
Methotrexate
Protease inhibitors
Indinavir
Nelfinavir
Ritonavir
Antibiotics
Erythromycin
Rifampicin
Cardiac drugs
Digoxin
Quinidine
Lovastatin
Antihistamines
Terfenadine
Others
Domperidone
Loperamide

Table 3 Studies on the effects of corticosteroids on multidrug resistance/P-glycoprotein 170 expression

Author	Subject	Dexamethasone treated	Effects
Zhao ¹⁴	Mouse hepatoma cell line	Yes	↑mdr1 and mdr3; ↔ mdr2
	Human hepatoma cell line	Yes	↑MDR1
	HeLa cell line	Yes	↔MDR1
	Rat liver	Yes	↑mdr2 only in male rats; ↔mdr1 and mdr3 in either sex
Lin ⁴⁰	Rat liver and intestines	Yes	↑P-gp both liver and intestines
Demeule ⁴¹	Rat organs	Yes	↑P-gp in lungs and liver, ↓in kidneys, ?in intestines
Murakami ⁴²	Rat intestines	Yes	↑P-gp expression and function
	Rat intestines (induced acute liver and renal failure)	No	↑P-gp expression but ↓function

↑, increased; ↔, no change; ↓, decreased;
P-gp, P-glycoprotein 170.

P-glycoprotein in the adrenal glands and endometrium suggest a role in the transport of steroid related compounds. In addition, these compounds are all substrates of P-glycoprotein. Nevertheless, at the present time, the available literature is complex and inconsistent (table 3).

In mouse hepatoma cell cultures treated with dexamethasone, Zhao *et al* demonstrated induction of mdr1 and mdr3 but not mdr2 transcripts. Similarly, MDR1 mRNA levels were elevated in a dexamethasone treated human hepatoma line. This effect was not seen in a non-hepatoma cell line (HeLa).¹⁴ Several studies have investigated the effect of corticosteroids in animal models. In a study examining expression of CYP3A and mdr in rat liver, dexamethasone was found to induce mdr2 expression only in male livers. Curiously, *mdr1a* and *1b* were not induced in either sex.³⁹ Lin *et al* showed that dexamethasone induces P-glycoprotein in the intestines and liver of rats with the functional effect of decreased plasma substrate level (indinavir).⁴⁰ However, Demeule *et al* found no increase in intestinal expression. In these animals, expression was upregulated in the lungs and liver whereas an opposite effect was observed in kidney tissue.⁴¹

Although P-glycoprotein expression may be influenced by corticosteroids or other pharmacological agents, recent data illustrate that the level of expression may not necessarily correlate with its function. Murakami *et al* demonstrated that although increased P-glycoprotein expression was evident in rats with induced acute renal and liver failure, in vivo P-glycoprotein activity was significantly depressed. Plasma from these diseased rats (with renal and liver failure) demonstrated a significantly greater inhibitory effect on P-glycoprotein function (in a Caco-2 cell line) compared with dexamethasone treated rats.⁴² It is hypothesised that in disease states, endogenous related compounds such as corticosterone (a P-glycoprotein inhibitor) may participate to suppress MDR1 activity.

Another intriguing aspect of the pharmacological regulation of expression of P-glycoprotein is seen in the multidrug resistance phenomenon. This is observed in tumour cells which, following initial contact with one anticancer agent, become rapidly resistant to other unrelated agents. This phenotype is mainly due to overexpression of the MDR1 gene. One possible mechanism for this may be through gene rearrangement. Mickley *et al* found that in several drug resistant cancer cell lines as well as samples from two leukaemic patients who had developed drug resistance, gene rearrangement had occurred resulting in activation or increased expression of MDR1.⁴³ Although a feasible hypothesis, there is no evidence to support selection of aberrant clones of the MDR gene as the cause of the development of multidrug resistance.^{44 45}

The above studies suggest that regulation of MDR1 gene expression is likely to be gene, cell, and perhaps gender specific. Furthermore, future work in this area will have to

consider not only expression but also the function of P-glycoprotein.

Genetic influences on MDR

The MDR1 gene lies on chromosome 7q21.1. Several polymorphisms of the gene have been characterised (table 4).⁴⁶ Hoffmeyer *et al* first described a single base polymorphism in exon 26 (C3435T) and suggested that this variant influences P-glycoprotein expression in the duodenum and P-glycoprotein activity.⁴⁷ In this study, healthy individuals homozygous for this variant (TT genotype) had significantly lower duodenal expression of P-glycoprotein and higher serum digoxin levels. Moreover, evaluation of maximum plasma concentrations during steady state digoxin administration showed a statistically significant increase (38%) in the homozygous TT genotype compared with the CC genotype.

It is particularly noteworthy that the MDR1 C3435T allelic variant, a single nucleotide polymorphism (SNP), is non-coding and does not affect amino acid sequence change. Although a number of coding mutations within the MDR1 gene (table 4) have been identified, none of these variants is in strong linkage disequilibrium with the C3435T variant.^{46 48 49} The observed functional effect ascribed to the C3435T variant may none the less reflect linkage disequilibrium with a polymorphism elsewhere in the genome that modifies MDR1 expression and function. However, at present this hypothesis requires further detailed studies of the gene. In parallel studies, Kimchi-Sarfaty *et al* demonstrated that there were no functional consequences of the five commoner amino acid altering SNPs (including 2677 SNP) in this gene, on both cellular localisation and transport function of P-glycoprotein.⁵⁰

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This exonic variant (C3435T) has received considerable attention although controversy still exists regarding the functional effect of this polymorphism. Of the five published studies investigating the effect of this polymorphism on expression of MDR1, three studies reported decreased expression and two studies increased expression in association with this SNP (table 5). Kim *et al* showed that the MDR 1*2 haplotype (G2677T/C3435T) was associated with increased P-glycoprotein function in vitro and low concentrations of a known substrate, fexofenadine.⁵¹ Fellay *et al* demonstrated that the MDR1 3435 TT genotype was associated with low expression of MDR1 transcripts and P-glycoprotein expression

Table 4 Single nucleotide polymorphisms (SNPs) in the MDR1 gene

SNP	Region	n	Heterozygous (%)	Homozygous (%)	Estimated	Effect
T-12C	Exon 1	85	11.8	0	0.4	Non-coding
G-1A	Exon 2	188	11.2	0	0.4	Translation
A61G	Exon 2	188	17.6	0.5	0.81	Asn21Asp
G-25T	Intron 4	85	26.0	3.5	2.3	Unknown
G-35C	Intron 4	85	1.2	0	0.01	Unknown
T307C	Exon 5	85	1.2	0	0.01	Phe103Leu
C+139T	Intron 5	85	48.2	16.5	16.8	Unknown
C+145T	Intron 5	85	2.4	0	0.01	Unknown
G1199A	Exon 11	85	12.9	0	0.4	Ser400Asn
C1236T	Exon 12	188	48.9	13.3	14.4	Gly412Gly
C+44T	Intron 12	188	11.7	0	0.4	Unknown
T-76A	Intron 16	85	45.9	22.4	20.3	Unknown
A+137G	Intron 17	85	1.2	0	0.01	Unknown
G2677T	Exon 21	83	43.4	42.2	38.4	Ala893Ser
G2995A	Exon 24	36	11.1	0	0	Ala999Thr
C3435T	Exon 26	537	47.7	26.4	24.1	Ile1145Ile
C3396T	Exon 26	188	0.53	0	0.01	Wobble

The positions of the identified SNPs correspond to positions of the MDR1 cDNA (gb: M14758, codon TTC in exon 10) with the first base of the ATG start codon set to 1. SNPs that are located in introns are presented as exon +/–n, where n=nucleotide upstream (+) or downstream (–) of exons. The predicted ratios of homozygous genotypes (q²) was calculated on the basis of Hardy-Weinberg distribution.

in peripheral blood mononuclear cells with low plasma levels of nelfinavir and efavirenz (antiretroviral agents).³² Hitzl *et al* showed that in CD56⁺ cells (natural killer cells), the MDR1 3435 TT genotype was associated with lower P-glycoprotein function and expression compared with the CC genotype.³³ In another study, Nakamura *et al* investigated the effect of the C3435T mutation on the expression level of MDR1 mRNA in duodenal enterocytes of healthy Japanese subjects³⁴ and found significantly elevated levels of transcripts in those patients with the TT compared with the CC allele. Given the likely complicated interactions with the cytochrome P450 system and the presence of other poorly characterised transporter systems, the relationship between the function of P-glycoprotein and drug substrate levels measured in vivo or in vitro is debatable. This consideration and the differences in study designs may explain these apparently contradictory results. Moreover, there may be cell specific differences in regulation. There is a clear need for further well designed studies using in vivo and ex vivo systems to reconcile these differences.

The MDR1 gene lies within the region of chromosome 7q which a recent two stage genome wide search has implicated as a putative susceptible locus for the development of IBD.²¹ The effects of polymorphisms within this gene on intestinal expression suggest that MDR1 gene and function may be important determinants of the development of IBD or of disease behaviour. This hypothesis is even more attractive given the effects of targeted gene deletion in mice, as described previously.²² Current investigations will provide further insights into this area.

MDR1 AND INFLAMMATORY BOWEL DISEASE

The finding of the development of spontaneous colitis in genetically engineered *mdr1a*–/– mice might suggest that

reduced MDR1 expression or activity in intestinal epithelial cells may be a factor in the pathogenesis of IBD. However, at present, there is surprisingly little published literature to investigate this hypothesis in humans. Two studies have focused on MDR1 expression in IBD.^{35, 36}

Farrell *et al* investigated the hypothesis that altered P-glycoprotein expression on lymphocytes may predict responsiveness to medical therapy.³⁵ Corticosteroids and cyclosporin are both substrates of P-glycoprotein.³⁷ Peripheral blood lymphocytes (PBL) from patients with active Crohn's disease (15 patients) and ulcerative colitis (28 patients) in whom medical therapy had failed and surgical intervention had been necessary were shown to have higher expression of P-glycoprotein compared with those from patients who had inactive disease and required surgery for obstruction (25 patients) or dysplasia (13 patients) ($p < 0.0001$ and 0.0002 , respectively). Interestingly, in ulcerative colitis, there was no significant difference when PBL P-glycoprotein expression was compared between patients requiring colectomy for failed medical treatment and the group with active ulcerative colitis ($p = 0.6$). Farrell *et al* went on to demonstrate that by blocking the action of P-glycoprotein on human cell lines, higher intracellular levels of cyclosporin and corticosteroids were achieved³⁸ and speculated that this may be a potential target for pharmacotherapy.

In Farrell's initial study,³⁵ MDR1 expression of the intestinal epithelial cells in inflamed areas of colon in patients with active ulcerative colitis ($n = 6$) was not different from expression in non-inflamed areas. Comparisons were not made with normal controls and it remains to be evaluated whether altered levels of MDR1 expression in the colon in humans lead to increased susceptibility to the development of IBD (as observed in studies involving mice models lacking the *mdr1a* gene). Although, intraepithelial lymphocytes/colonic

Table 5 Cell populations and transporter substances used in functional studies of MDR1 3435TT genotype

Study	Cell type	Expression/function MDR1 3435T	Plasma drug concentrations
Hoffmeyer ⁴⁷	Enterocytes	↓MDR1 mRNA	↑Digoxin
Fellay ⁵²	Peripheral blood mononuclear cells	↓MDR1 mRNA and Pgp expression	↓Nelfinavir and efavirvir
Kim ⁵¹	Transfected murine NIH-3T3	↑Pgp function in vitro for MDR1 *2 haplotype	↓Fexofenadine
Hitzl ⁵³	CD 56 natural killer cells	↓MDR1 mRNA and Pgp expression	NA
Nakamura ⁵⁴	Duodenal enterocytes	↑MDR1 mRNA	↓Digoxin

Pgp, P-glycoprotein; MDR1 *2 haplotype, G677T/C3435T; NA, not available, MDR, multidrug resistance; mRNA, messenger RNA.
↓, decrease; ↑, increase.

epithelial expression of MDR1 correlated significantly with PBL expression, overall expression in the control group was in fact similar to groups of patients with inactive ulcerative colitis/Crohn's disease.

With respect to MDR1 overexpression as a factor in corticosteroid resistance, this has also been studied in other conditions. In patients with glucocorticoid resistant asthma, P-glycoprotein expression on PBL was not found to be increased compared with those with responsive or mild asthma.⁵⁹ In patients with rheumatoid arthritis treated with glucocorticoids, expression of P-glycoprotein on PBL was increased compared with those without therapy.⁶⁰ Patients with rheumatoid arthritis without corticosteroid therapy did not demonstrate higher expression of MDR1 and this again may reflect a secondary effect to corticosteroids. In a subset of patients with IBD not requiring surgery (n=72), multiple regression analysis of several clinical variables showed that only concurrent corticosteroid therapy was significantly associated with high PBL MDR1 expression (p=0.001).⁵⁵

Intraepithelial MDR1 expression and function were suggested to be lower in ulcerative colitis compared with Crohn's disease and healthy controls

There are several caveats to these studies. Firstly, circulating lymphocytes consist of different subsets of lymphocytes which express MDR1 at varying levels.⁶¹ Indeed, within the gastrointestinal tract it is now well established that lymphocytes homing to the gut associated mucosal lymphoid tissue are phenotypically distinct from circulatory lymphocytes. It is also pertinent that Yacyshyn *et al* have demonstrated that the intraepithelial, lamina propria, and PBL all demonstrate different levels of MDR1 expression and activity in ulcerative colitis, Crohn's disease, and healthy controls. In this study, CD3 and CD8 lymphocytes were also present in different proportions in these groups of subjects. These subsets were also shown to have intrinsically different levels of P-glycoprotein function (CD8 has higher levels of P-glycoprotein). Overall, intraepithelial MDR1 expression and function were suggested by these investigators to be lower in ulcerative colitis compared with Crohn's disease and healthy controls.⁵⁶

Secondly, the effect of corticosteroids (or indeed other treatments used in IBD) on MDR1 expression is not fully established. It remains to be seen whether higher expression of P-glycoprotein reflects a secondary phenomenon to corticosteroid therapy or a primary one, influencing the response to treatment. Finally, as discussed earlier, increased expression does not necessarily imply increased function in diseased states.

MDR1 AND COLORECTAL CANCER

P-glycoprotein expression appears to be highest in tumours originating from normal tissues expressing P-glycoprotein^{13 10} which include colorectal epithelium. In studies of colorectal carcinoma, expression of P-glycoprotein correlated with pathological grading of tumours, being most intense in well differentiated tumours and low in poorly differentiated ones.⁶²

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In view of the presence of intrinsic drug resistance to natural products of anticancer drugs, the high P-glycoprotein expression reported in colorectal cancer, and in vitro studies demonstrating successful P-glycoprotein reversal in colon cancer cell line, specific inhibition of P-glycoprotein has been

carried out in clinical trials to determine whether a more favourable response to chemotherapy is achieved. As yet the results have been disappointing and inconclusive. The reasons for this appear to be a combination of drug toxicity of the resistance modifying agents together with an inability to interpret and compare results in different studies as a consequence of the lack of standardisation in the measurement of P-glycoprotein expression.⁶³⁻⁶⁵

P-glycoprotein expression has been associated with a poor prognosis in neuroblastoma, sarcoma, and acute myeloid leukaemia.^{66 67} Its prognostic value in colorectal cancer is less clear. Weinstein *et al* reported a significant correlation between the presence of P-glycoprotein positive tumour cells at the leading edge of the tumour and an increased incidence of tumour vessel invasion and lymph node metastases.⁶⁸ Another study reported P-glycoprotein to have prognostic value in Dukes' stage B colon cancers. However, this study was small (52 patients) and the p value was of marginal significance.⁶⁹ Three other studies failed to demonstrate a relationship between P-glycoprotein expression and shorter (disease free) survival.⁷⁰⁻⁷²

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An intriguing study has identified the MDR1 gene as a target of transactivation by the T cell factor 4/ β -catenin complex in early colorectal carcinogenesis.⁷³ Mutational inactivation of the tumour suppressor gene APC results in accumulation of cytoplasmic β -catenin through failure of degradation.⁷⁴ β -Catenin forms a complex with T cell factor 4 which acts as a transcription factor. Target genes have included c-myc, cyclin D1, matrilysin, T cell factor 1, and peroxisome proliferator activated receptors which are all implicated in the development of colorectal cancer.⁷⁵⁻⁸⁰

Colorectal cell lines that are mutant for APC or β -catenin have high levels of T cell factor mediated transcription which is thought to be the basis of tumorigenesis. Yamada *et al* performed a large scale comparison of gene expression by involving cDNA microarray technology in the colorectal cell line DLD1 in which T cell factor 4/ β catenin transactivation had been suppressed and gene expression data were compared with wild-type DLD1 cells.⁸¹ More than 7000 genes were studied, and MDR1 was found to be transcriptionally downregulated after inactivation of T cell factor 4. It is clearly pertinent that the promoter region of the MDR1 gene contains several T cell factor 4 binding sequences. The authors subsequently demonstrated that in adenomatous polyps from patients with familial adenomatous polyposis syndrome, MDR1 expression was significantly increased and correlated with β -catenin.

This study thus provided strong evidence of a link between colorectal cancer and the MDR1 gene. Further functional studies are necessary to establish the functional significance of these findings. It is hypothesised that overexpression of MDR1 may influence apoptotic pathways by secreting endogenous or xenobiotic toxic substances into the lumen or by mediating a more generic antiapoptotic function. The MDR1 gene product, P-glycoprotein, protects the cell against a wide variety of caspase dependent death stimuli including gas ligand, tumour necrosis factor, and ultraviolet irradiation.⁸²

"The MDR1 gene may have an important role in the tumorigenesis of colorectal cancer"

A number of in vitro studies have focused on the effect of the tumour suppressor gene p53 on expression of the MDR1 gene. These report an inhibitory role of the wild-type p53 on the MDR1 promoter region whereas mutant p53 variants act

as activators, probably due to loss of its inhibitory role.^{83–86} Together with the T cell factor elements described earlier, these may represent only a relatively small contribution to the overall complex transcriptional regulation of the MDR1 gene by other transactivating genes. However, it has become apparent that the MDR1 gene may have an important role in the tumorigenesis of colorectal cancer.

CONCLUSION

There is now strong evidence that the gene product of MDR plays a critical role in host-bacterial interactions in the gastrointestinal tract and maintenance of intestinal homeostasis. Perhaps the most important current challenge is to define the physiological role of MDR1 in the healthy intestine. Expression and function of the MDR1 gene in common gastrointestinal diseases is the subject of detailed evaluation, and progress in understanding the pathogenesis of chronic IBD and colorectal cancer may result from these studies.

Moreover, there is real hope that further studies of the MDR1 gene may lead to direct clinical application, particularly in the field of pharmacogenomics. The possibility of predicting the responsiveness of IBD or colorectal cancer to therapy by measuring P-glycoprotein expression or allelic variation is exciting and appears increasingly realistic. Measurement of this and other subclinical markers may help in optimising the use of current and novel therapeutic agents.^{55–87}

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REFERENCES

- Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* 1976;**455**:152–62.
- Hsu SI, Lothstein L, Horwitz SB. Differential overexpression of three mdr gene family members in multidrug-resistant J774.2 mouse cells. Evidence that distinct P-glycoprotein precursors are encoded by unique mdr genes. *J Biol Chem* 1989;**264**:12053–62.
- Chen CJ, Chin JE, Ueda K, et al. Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells. *Cell* 1986;**47**:381–9.
- Ueda K, Clark DP, Chen CJ, et al. The human multidrug resistance (mdr1) gene. cDNA cloning and transcription initiation. *J Biol Chem* 1987;**262**:505–8.
- Fardel O, Payen L, Courtois A, et al. Regulation of biliary drug efflux pump expression by hormones and xenobiotics. *Toxicology* 2001;**167**:37–46.
- Smit JJ, Schinkel AH, Oude ER, et al. Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. *Cell* 1993;**75**:451–62.
- Deleuze JF, Jacquemin E, Dubuisson C, et al. Defect of multidrug-resistance 3 gene expression in a subtype of progressive familial intrahepatic cholestasis. *Hepatology* 1996;**23**:904–8.
- Jacquemin E. Progressive familial intrahepatic cholestasis. Genetic basis and treatment. *Clin Liver Dis* 2000;**4**:753–63.
- Nagata K, Nishitani M, Matsuo M, et al. Nonequivalent nucleotide trapping in the two nucleotide binding folds of the human multidrug resistance protein MRP1. *J Biol Chem* 2000;**275**:17626–30.
- Thiebaut F, Tsuruo T, Hamada H, et al. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci U S A* 1987;**84**:7735–8.
- Wijnholds J, Evers R, van Leusden MR, et al. Increased sensitivity to anticancer drugs and decreased inflammatory response in mice lacking the multidrug resistance-associated protein. *Nat Med* 1997;**3**:1275–9.
- Rao VV, Dahlheimer JL, Bardgett ME, et al. Choroid plexus epithelial expression of MDR1 P glycoprotein and multidrug resistance-associated protein contribute to the blood-cerebrospinal-fluid drug-permeability barrier. *Proc Natl Acad Sci U S A* 1999;**96**:3900–5.
- Cordon-Cardo C, O'Brien JP, Casals D, et al. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci U S A* 1989;**86**:695–8.
- Zhao JY, Ikeguchi M, Eckersberg T, et al. Modulation of multidrug resistance gene expression by dexamethasone in cultured hepatoma cells. *Endocrinology* 1993;**133**:521–8.
- Klimecki WT, Futscher BW, Grogan TM, et al. P-glycoprotein expression and function in circulating blood cells from normal volunteers. *Blood* 1994;**83**:2451–8.
- Chianale J, Vollrath V, Wielandt AM, et al. Differences between nuclear run-off and mRNA levels for multidrug resistance gene expression in the cephalocaudal axis of the mouse intestine. *Biochim Biophys Acta* 1995;**1264**:369–76.
- Fricker G, Drewe J, Huwyler J, et al. Relevance of p-glycoprotein for the enteral absorption of cyclosporin A: in vitro-in vivo correlation. *Br J Pharmacol* 1996;**118**:1841–7.
- Fojo AT, Ueda K, Slamon DJ, et al. Expression of a multidrug-resistance gene in human tumors and tissues. *Proc Natl Acad Sci U S A* 1987;**84**:265–9.
- Yumoto R, Murakami T, Sanemasa M, et al. Pharmacokinetic interaction of cytochrome P450 3A-related compounds with rhodamine 123, a P-glycoprotein substrate, in rats pretreated with dexamethasone. *Drug Metab Dispos* 2001;**29**:145–51.
- Treize AE, Romano PR, Gill DR, et al. The multidrug resistance and cystic fibrosis genes have complementary patterns of epithelial expression. *EMBO J* 1992;**11**:4291–303.
- Satsangi J, Parkes M, Louis E, et al. Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nat Genet* 1996;**14**:199–202.
- Panwala CM, Jones JC, Viney JL. A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis. *J Immunol* 1998;**161**:5733–44.
- Schinkel AH, Mayer U, Wagenaar E, et al. Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. *Proc Natl Acad Sci U S A* 1997;**94**:4028–33.
- Shomer NH, Dangler CA, Marini RP, et al. Helicobacter bilis/Helicobacter rodentium co-infection associated with diarrhea in a colony of scid mice. *Lab Anim Sci* 1998;**48**:455–9.
- Cahill RJ, Foltz CJ, Fox JG, et al. Inflammatory bowel disease: an immunity-mediated condition triggered by bacterial infection with Helicobacter hepaticus. *Infect Immun* 1997;**65**:3126–31.
- Burich A, Hershberg R, Waggie K, et al. Helicobacter-induced inflammatory bowel disease in IL-10- and T cell-deficient mice. *Am J Physiol Gastrointest Liver Physiol* 2001;**281**:G764–78.
- Cahill RJ, Foltz CJ, Fox JG, et al. Inflammatory bowel disease: an immunity-mediated condition triggered by bacterial infection with Helicobacter hepaticus. *Infect Immun* 1997;**65**:3126–31.
- Gewirtz AT, Navas TA, Lyons S, et al. Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. *J Immunol* 2001;**167**:1882–5.
- Neish AS, Gewirtz AT, Zeng H, et al. Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. *Science* 2000;**289**:1560–3.
- Sparreboom A, van Asperen J, Mayer U, et al. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. *Proc Natl Acad Sci U S A* 1997;**94**:2031–5.
- Mayer U, Wagenaar E, Beijnen JH, et al. Substantial excretion of digoxin via the intestinal mucosa and prevention of long-term digoxin accumulation in the brain by the mdr1a P-glycoprotein. *Br J Pharmacol* 1996;**119**:1038–44.
- Benet LZ, Cummins CL. The drug efflux-metabolism alliance: biochemical aspects. *Adv Drug Deliv Rev* 2001;**50**(suppl 1):S3–11.
- Labialle S, Gayet L, Marthinet E, et al. Transcriptional regulators of the human multidrug resistance 1 gene: recent views. *Biochem Pharmacol* 2002;**64**:943–8.
- Shapiro AB, Fox K, Lam P, et al. Stimulation of P-glycoprotein-mediated drug transport by prazosin and progesterone. Evidence for a third drug-binding site. *Eur J Biochem* 1999;**259**:841–50.
- Greiner B, Eichelbaum M, Fritz P, et al. The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J Clin Invest* 1999;**104**:147–53.
- Asghar A, Gorski JC, Haehner-Daniels B, et al. Induction of multidrug resistance-1 and cytochrome P450 mRNAs in human mononuclear cells by rifampin. *Drug Metab Dispos* 2002;**30**:20–6.
- Westphal K, Weinbrenner A, Zschiesche M, et al. Induction of P-glycoprotein by rifampin increases intestinal secretion of talinolol in human beings: a new type of drug/drug interaction. *Clin Pharmacol Ther* 2000;**68**:345–55.
- Geick A, Eichelbaum M, Burk O. Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J Biol Chem* 2001;**276**:14581–7.
- Salphati L, Benet LZ. Modulation of P-glycoprotein expression by cytochrome P450 3A inducers in male and female rat livers. *Biochem Pharmacol* 1998;**55**:387–95.
- Lin JH, Chiba M, Chen IW, et al. Effect of dexamethasone on the intestinal first-pass metabolism of indinavir in rats: evidence of cytochrome P-450 3A (correction of P-450 A) and p-glycoprotein induction. *Drug Metab Dispos* 1999;**27**:1187–93.
- Demeule M, Jodoin J, Beaulieu E, et al. Dexamethasone modulation of multidrug transporters in normal tissues. *FEBS Lett* 1999;**442**:208–14.
- Murakami T, Yumoto R, Nagai J, et al. Factors affecting the expression and function of P-glycoprotein in rats: drug treatments and diseased states. *Pharmazie* 2002;**57**:102–7.
- Mickley LA, Spengler BA, Knutsen TA, et al. Gene rearrangement: a novel mechanism for MDR-1 gene activation. *J Clin Invest* 1997;**99**:1947–57.
- Van Den Heuvel-Eibrink MM, Wiemer EA, de Boevere MJ, et al. MDR1 gene-related clonal selection and P-glycoprotein function and

- expression in relapsed or refractory acute myeloid leukemia. *Blood* 2001;**97**:3605–11.
- 45 **van der Kolk DM**, de Vries EG, Noordhoek L, *et al.* Activity and expression of the multidrug resistance proteins P-glycoprotein, MRP1, MRP2, MRP3 and MRP5 in de novo and relapsed acute myeloid leukemia. *Leukemia* 2001;**15**:1544–53.
 - 46 **Cascorbi I**, Gerloff T, John A, *et al.* Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clin Pharmacol Ther* 2001;**69**:169–74.
 - 47 **Hoffmeyer S**, Burk O, von Richter O, *et al.* Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci U S A* 2000;**97**:3473–8.
 - 48 **Drescher S**, Schaeffeler E, Hitzl M, *et al.* MDR1 gene polymorphisms and disposition of the P-glycoprotein substrate fexofenadine. *Br J Clin Pharmacol* 2002;**53**:526–34.
 - 49 **Tang K**, Ngoi SM, Gwee PC, *et al.* Distinct haplotype profiles and strong linkage disequilibrium at the MDR1 multidrug transporter gene locus in three ethnic Asian populations. *Pharmacogenetics* 2002;**12**:437–50.
 - 50 **Kimchi-Sarfaty C**, Gribar JJ, Gottesman MM. Functional characterization of coding polymorphisms in the human MDR1 gene using a vaccinia virus expression system. *Mol Pharmacol* 2002;**62**:1–6.
 - 51 **Kim RB**, Leake BF, Choo EF, *et al.* Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther* 2001;**70**:189–99.
 - 52 **Fellay J**, Marzolini C, Meaden ER, *et al.* Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. *Lancet* 2002;**359**:30–6.
 - 53 **Hitzl M**, Drescher S, van der Kuip H, *et al.* The C3435T mutation in the human MDR1 gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD56+ natural killer cells. *Pharmacogenetics* 2001;**11**:293–8.
 - 54 **Nakamura T**, Sakaeda T, Horinouchi M, *et al.* Effect of the mutation [C3435T] at exon 26 of the MDR1 gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. *Clin Pharmacol Ther* 2002;**71**:297–303.
 - 55 **Farrell RJ**, Murphy A, Long A, *et al.* High multidrug resistance [P-glycoprotein 170] expression in inflammatory bowel disease patients who fail medical therapy. *Gastroenterology* 2000;**118**:279–88.
 - 56 **Yacyszyn B**, Maksymowych W, Bowen-Yacyszyn MB. Differences in P-glycoprotein-170 expression and activity between Crohn's disease and ulcerative colitis. *Hum Immunol* 1999;**60**:677–87.
 - 57 **Ueda K**, Okamura N, Hirai M, *et al.* Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J Biol Chem* 1992;**267**:24248–52.
 - 58 **Farrell RJ**, Menconi MJ, Keates AC, *et al.* P-Glycoprotein-170 inhibition significantly reduces cortisol and ciclosporin efflux from human intestinal epithelial cells and T lymphocytes. *Aliment Pharmacol Ther* 2002;**16**:1021–31.
 - 59 **Montano E**, Schmitz M, Blaser K, *et al.* P-glycoprotein expression in circulating blood leukocytes of patients with steroid-resistant asthma. *J Invest Allergol Clin Immunol* 1996;**6**:14–21.
 - 60 **Maillefert JF**, Maynadie M, Tebib JG, *et al.* Expression of the multidrug resistance glycoprotein 170 in the peripheral blood lymphocytes of rheumatoid arthritis patients. The percentage of lymphocytes expressing glycoprotein 170 is increased in patients treated with prednisolone. *Br J Rheumatol* 1996;**35**:430–5.
 - 61 **Ludescher C**, Pall G, Irshick EU, *et al.* Differential activity of P-glycoprotein in normal blood lymphocyte subsets. *Br J Haematol* 1998;**101**:722–7.
 - 62 **Potocnik U**, Ravnik-Glavac M, Golouh R, *et al.* Naturally occurring mutations and functional polymorphisms in multidrug resistance 1 gene: correlation with microsatellite instability and lymphoid infiltration in colorectal cancers. *J Med Genet* 2002;**39**:340–6.
 - 63 **Kornek G**, Schenk T, Raderer M, *et al.* Tissue polypeptide-specific antigen (TPS) in monitoring palliative treatment response of patients with gastrointestinal tumours. *Br J Cancer* 1995;**71**:182–5.
 - 64 **Scheithauer W**, Schenk T, Czejk M. Pharmacokinetic interaction between epirubicin and the multidrug resistance reverting agent D-verapamil. *Br J Cancer* 1993;**68**:8–9.
 - 65 **Czejk M**, Schenk T, Scheithauer W. The combination of epirubicin with d-verapamil and/or tamoxifen in cell cultures. *Pharmazie* 1993;**48**:148–9.
 - 66 **Van Den Heuvel-Eibrink MM**, Sonneveld P, Pieters R. The prognostic significance of membrane transport-associated multidrug resistance (MDR) proteins in leukemia. *Int J Clin Pharmacol Ther* 2000;**38**:94–110.
 - 67 **Chan HS**, Haddad G, Thorner PS, *et al.* P-glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. *N Engl J Med* 1991;**325**:1608–14.
 - 68 **Weinstein RS**, Jakate SM, Dominguez JM, *et al.* Relationship of the expression of the multidrug resistance gene product (P-glycoprotein) in human colon carcinoma to local tumor aggressiveness and lymph node metastasis. *Cancer Res* 1991;**51**:2720–6.
 - 69 **Sinicrope FA**, Hart J, Brasitus TA, *et al.* Relationship of P-glycoprotein and carcinoembryonic antigen expression in human colon carcinoma to local invasion, DNA ploidy, and disease relapse. *Cancer* 1994;**74**:2908–17.
 - 70 **Ando Y**, Tsuchiya A, Oki S, *et al.* Flow cytometric DNA analysis of malignant potential in colorectal cancer. *Gan To Kagaku Ryoho* 1996;**23**(suppl 2):112–7.
 - 71 **Pirker R**, Wallner J, Gsur A, *et al.* MDR1 gene expression in primary colorectal carcinomas. *Br J Cancer* 1993;**68**:691–4.
 - 72 **Wallner J**, Depisch D, Gsur A, *et al.* MDR1 gene expression and its clinical relevance in primary gastric carcinomas. *Cancer* 1993;**71**:667–71.
 - 73 **Yamada T**, Takaoka AS, Naishiro Y, *et al.* Transactivation of the multidrug resistance 1 gene by T-cell factor 4/beta-catenin complex in early colorectal carcinogenesis. *Cancer Res* 2000;**60**:4761–6.
 - 74 **Munemitsu S**, Albert I, Souza B, *et al.* Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. *Proc Natl Acad Sci U S A* 1995;**92**:3046–50.
 - 75 **He TC**, Chan TA, Vogelstein B, *et al.* PPARdelta is an APC-regulated target of nonsteroidal anti-inflammatory drugs. *Cell* 1999;**99**:335–45.
 - 76 **He TC**, Sparks AB, Rago C, *et al.* Identification of c-MYC as a target of the APC pathway. *Science* 1998;**281**:1509–12.
 - 77 **Ougolkov AV**, Yamashita K, Mai M, *et al.* Oncogenic beta-catenin and MMP-7 (matrilysin) cosegregate in late-stage clinical colon cancer. *Gastroenterology* 2002;**122**:60–71.
 - 78 **Davies G**, Jiang WG, Mason MD. Matrilysin mediates extracellular cleavage of E-cadherin from prostate cancer cells: a key mechanism in hepatocyte growth factor/scatter factor-induced cell-cell dissociation and in vitro invasion. *Clin Cancer Res* 2001;**7**:3289–97.
 - 79 **Brabletz T**, Jung A, Dag S, *et al.* beta-Catenin induces invasive growth by activating matrix metalloproteinases in colorectal carcinoma. *Verh Dtsch Ges Pathol* 2000;**84**:175–81.
 - 80 **Arber N**, Hibshoosh H, Moss SF, *et al.* Increased expression of cyclin D1 is an early event in multistage colorectal carcinogenesis. *Gastroenterology* 1996;**110**:669–74.
 - 81 **Yamada T**, Takaoka AS, Naishiro Y, *et al.* Transactivation of the multidrug resistance 1 gene by T-cell factor 4/beta-catenin complex in early colorectal carcinogenesis. *Cancer Res* 2000;**60**:4761–6.
 - 82 **Smyth MJ**, Johnstone RW, Cretny E, *et al.* Multiple deficiencies underlie NK cell inactivity in lymphotoxin-alpha gene-targeted mice. *J Immunol* 1999;**163**:1350–3.
 - 83 **Bush JA**, Li G. Regulation of the Mdr1 isoforms in a p53-deficient mouse model. *Carcinogenesis* 2002;**23**:1603–7.
 - 84 **Sampath J**, Sun D, Kidd VJ, *et al.* Mutant p53 cooperates with ETS and selectively up-regulates human MDR1 not MRP1. *J Biol Chem* 2001;**276**:39359–67.
 - 85 **Nguyen KT**, Liu B, Ueda K, *et al.* Transactivation of the human multidrug resistance (MDR1) gene promoter by p53 mutants. *Oncol Res* 1994;**6**:71–7.
 - 86 **Chin KV**, Ueda K, Pastan I, *et al.* Modulation of activity of the promoter of the human MDR1 gene by Ras and p53. *Science* 1992;**255**:459–62.
 - 87 **Vermeire S**, Louis E, Rutgeerts P, *et al.* NOD2/CARD15 does not influence response to infliximab in Crohn's disease. *Gastroenterology* 2002;**123**:106–11.