Decreased functional activity of multidrug resistance protein in primary colorectal cancer

Tamás Micsik1,2*, András Lőrincz2,8, Tamás Mersich3, Zsolt Baranyai4,9, István Besznyák Jr3, Kristóf Dede3, Attila Zaránd3,9, Ferenc Jakab3, László Krecsák Szőllősi5, György Kérí2,6, Richard Schwab2,7 and István Peták2,6,7

Abstract

Background: The ATP-Binding Cassette (ABC)-transporter MultiDrug Resistance Protein 1 (MDR1) and Multidrug Resistance Related Protein 1 (MRP1) are expressed on the surface of enterocytes, which has led to the belief that these high capacity transporters are responsible for modulating chemosensitivity of colorectal cancer. Several immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR) studies have provided controversial results in regards to the expression levels of these two ABC-transporters in colorectal cancer. Our study was designed to determine the yet uninvestigated functional activity of MDR1 and MRP1 transporters in normal human enterocytes compared to colorectal cancer cells from surgical biopsies.

Methods: 100 colorectal cancer and 28 adjacent healthy mucosa samples were obtained by intraoperative surgical sampling. Activity of MDR1 and MRP1 of viable epithelial and cancer cells were determined separately with the modified calcein-assay for multidrug resistance activity and sufficient data of 73 cancer and 11 healthy mucosa was analyzed statistically.

Results: Significantly decreased mean MDR1 activity was found in primary colorectal cancer samples compared to normal mucosa, while mean MRP1 activity showed no significant change. Functional activity was not affected by gender, age, stage or grade and localization of the tumor.

Conclusion: We found lower MDR activity in cancer cells versus adjacent, apparently, healthy control tissue, thus, contrary to general belief, MDR activity seems not to play a major role in primary drug resistance, but might rather explain preferential/selective activity of Irinotecan and/or Oxaliplatin. Still, this picture might be more complex since chemotherapy by itself might alter MDR activity, and furthermore, today limited data is available about MDR activity of cancer stem cells in colorectal cancers.

Virtual slides: The virtual slide(s) for this article can be found here: http://www.diagnosticpathology.diagnomx.eu/vs/1675739129145824

Background

ABC-(ATP-Binding Cassette) transporters are transmembrane proteins expressed in the physiological barriers of the human body pumping out a high diversity of substrates (toxins, chemotherapeutics, medications, bile acids etc) from the cells and thus have important role in the detoxification of our body against xenobiotics. Activation of the same MDR-transporters of cancer cells can cause multidrug resistant phenomenon interfering with response to chemotherapy [1].

The clinically most important ABC-transporters are the MDR1 (MultiDrug Resistance protein 1, P-glycoprotein-170) having prognostic role in acute myeloid leukemia [2], sarcomas [3,4] and gallbladder carcinoma [5]; and MRP1 (Multidrug resistant Associated/Related Protein 1), which has prognostic relevance in neuroblastoma [6], hepatocellular carcinoma [7] and in non small cell lung cancer [8].

Based on the high expression of the ABC-transporters along the gastrointestinal tract [9] and the intrinsic low response rate of GI cancers to chemotherapy, colorectal cancer was thought to be chemoresistant due to MDR-proteins

* Correspondence: micsikt@gmail.com
11st Department of Pathology and Experimental Cancer Research, Semmelweis University, Úllői út 26, H-1085 Budapest, Hungary
2Rational Drug Design Laboratories, Cooperative Research Center, Semmelweis University, Úllői út 26, H-1085 Budapest, Hungary
Full list of author information is available at the end of the article

© 2015 Micsik et al; licensee BioMed Central. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
but later studies have not justified this theory [14-19]. The main therapy of colorectal cancer is the surgical resection of the tumor in combination with chemotherapeutic regimens were initially based on 5-fluorouracil (5FU - neither MDR1 nor MRP1 substrate), but recently Irinotecan (MDR1 substrate) and Oxaliplatin (MRP1 substrate) were also introduced in combination with monoclonal antibodies and resulted in better response-rate and survival rate even in metastatic cases [20,21].

As newer chemotherapeutics raised the possible role of MDR-transporters’ activity in response to therapy, we decided to study the functional activity of MDR1 and MRP1-proteins in freshly isolated viable colon carcinoma cells and normal epithelial cells with the modified calcein assay [22]. In our study of 73 cancer and 11 normal mucosa we found that MDR1 functional activity of colorectal cancer cells was decreased compared to normal enterocytes, while functional activity of MRP1 didn’t change significantly.

**Methods**

**Patient samples**

Clinical samples were obtained after approval by the national and local Ethical Committees at the Department of Surgery and Vascular Surgery of the Uzsoki Teaching Hospital, Budapest. All patients were enrolled after written consent and altogether 100 samples of primary colorectal cancer and 28 normal mucosal samples were taken into RPMI 1640 (11875-093, Gibco Invitrogen, Grand Island, NY) medium within 30 minutes after devascularization. Colon cases (n = 44) were chemotherapy naïve, while rectal cases (n = 29) received previous chemo-radiotherapy. The samples were stored at 4°C until being processed. Clinicopathological characteristics of the cases involved in the statistical analysis are shown in Table 1.

**Modified Calcein assay for solid tumors**

The samples were processed with the modified calcein assay [22]. Surgical samples were cut into small pieces, washed in HBSS buffer (14025-092, GibCO Invitrogen, Csertext, Budapest, Hungary) then incubated in 1 ml of 4 mg/ml collagenase (LS004212, Worthington collagenase type IV, Worthington Biochemical Corporation, NJ) while continuously mixing for 10 min at 37°C. The reaction was stopped by adding 200 μl 10% FBS (Foetal Bovine Serum – F-2442, Sigma-Aldrich, Budapest, Hungary). After filtering and washing the samples in HBSS, 600 μls of the yielded single-cell suspension were aliquoted into seven tubes.

The dual MDR1 and MRP1 inhibitor Verapamil (V4629, Sigma-Aldrich, Budapest, Hungary) was diluted in HBSS to 250 μM and 200 μl was added to three vials. The MRP1 inhibitor MK571 (340-021-M005, Alexis Biochemicals, Bio-Marker, Gödöllő, Hungary) was diluted in HBSS to 50 μM and 200 μl was added to another two vials. 200 μl HBSS buffer was added to the remaining two control vials. All samples were mixed gently, but thoroughly and subsequently 200 μl of 50 nM HBSS-diluted calcein-AM (C3100, Molecular Probes, Bio-Science, Budapest, Hungary) was added to each sample and incubated for exactly 10 minutes at 37°C. Samples were then rapidly chilled on ice for 5 minutes and spun down. Supernatant was discarded and cells were resuspended in 200 μl HBSS containing 2 μg/ml 7-AAD (AminoActiomyacinD – A9400, Sigma-Aldrich, Budapest, Hungary). 1 μg of isotype negative control mouse IgG1 (X093101-2, Dako-Frank Diagnosztika Kft., Budapest, Hungary) was added to one Verapamil treated sample and 1 μg of anti-BerEP4 mouse IgG1 antibody (M080401-2, Dako, Budapest, Hungary) to the other six samples. Subsequently, 0.5 μg of secondary Cy5 conjugated goat anti-mouse IgG antibody (115-175-003, Jackson Immuno Research, Izinta, Budapest, Hungary) was added to each sample and incubated in dark at room temperature for 30 minutes. Samples were spun down and resuspended in 200 μl HBSS containing 1 μg/ml 7-AAD and kept on ice until measurement.

Flow cytometric analysis was performed on Becton Dickinson FACSCalibur flow cytometer as shown in Figures 1, 2 and 3. Calcein signal was detected on FL-2 instead of the usual FL-1 for better electronic compensation (for details see [22]). Viable cells were gated and selected based on the positive calcein (FL-2) and negative 7-AAD (FL-3) signal of those and further analyzed on the FL4 (BerEp4 signal) and SSC diagram (Figure 1). BerEp4 negative cells were excluded with parallel gating of IgG negative control and BerEp4 samples (Figure 2) and calcein signal shifts of BerEp4 and calcein positive, but

---

**Table 1 Basic clinicopathological characteristics of studied primary ColoRectal Cancer cases included in statistical analysis**

|       | CRC = 73 | Average age | Mucosa = 11 |
|-------|----------|-------------|-------------|
| Males | 39       | 65,2        | 7           |
| Females | 34      | 68,6        | 4           |
| Right colon | Coecum:12 | Ascendens:8 |
| Left colon | Descendens:7 | Sigma:17 | Rectum:29 |
| Grade | Grade I9 | Grade II53 | Grade III11 |
| TNM Stage I22 | TNM Stage II21 | TNM Stage III11 | TNM Stage IV:19 |
| T1:5 | T1N0M04 | T2N0M1:10 | T2N1M0:18 |
| T3N0M18 | T3N1M0:9 | T3N1M1:6 |
| T4N0M13 | T4N1M1:9 |

pTNM version 6 was used during data collection. (CRC: ColoRectal Cancer; pTNM: pathological TNM-statifiction).
7AAD-negative cells were detected in each parallel samples (Figure 3).

Our assay used the Calcein-AM as a known substrate for both examined transporters. With the two transporter inhibitors (Verapamil for both MDR1 and MRP1 and MK571 only for the MRP1) the assay calculates the individual functional activity of both transporters as MAF-values (Multidrug Activity Factor) of the given sample. MAF values were calculated from the means of calcein fluorescence signals detected with the control HBSS and with the two inhibitors according to the mathematic formula: $\text{MAF}_{\text{Total}} = 100 \times \frac{F_{\text{Verapamil}} - F_{\text{HBSS}}}{F_{\text{Verapamil}}}$; $\text{MAF}_{\text{MRP1}} = 100 \times \frac{F_{\text{MK571}} - F_{\text{HBSS}}}{F_{\text{Verapamil}}}$; $\text{MAF}_{\text{MDR1}} = \text{MAF}_{\text{Total}} - \text{MAF}_{\text{MRP1}}$, where $F$ denotes the mean Calcein-fluorescence value determined as the average of the two parallel FL2 signals in the different samples. Samples with highly active MDR1 and MRP1 functional activity give MAF around 20-40, or higher, while samples without significant activity would show values of 0-5. Negative values are probable signs of other active transporters than MDR1 or MRP1.

The absolute number of all cells and viable cells, and the absolute number and percentage of viable epithelial cells were determined in each sample. The MAF-values were not affected by the cell number or cell viability or elapsed time from surgical sampling. Experiments yielding too few viable epithelial cells (under 100) were excluded, thus altogether 11 normal and 73 tumor samples could have been included in the statistical analysis.

Results were tested for normal distribution using the Kolmogorov-Smirnov test with Lilliefors significance correction. Homogeneity of variances was evaluated using the Levene test. For analysis of the variables that slightly derived from normal distribution and homoscedasticity,
the two-sample unequal-variance Student’s t-test was used [23], while other parameters were compared by equal variance t-test. Data are presented as mean +/- SD if normal, and median and inter-quartile range if non-normal. Data analysis was performed using the SPSS 17.0 software (SPSS Inc., Chicago, USA).

**Results**

There was a significant decrease (p = 0.03) in the MAF values of colorectal cancer cases (MAF: -7.80 ± 14.43) compared to the adjacent, apparently normal mucosa (MAF: 2.08 ± 11.17). This decrease was mainly due to the significant (p = 0.05) decrease in MAF of colorectal cancer cases (MAF: -3.9 ± 12.12) compared to normal mucosa (MAF: 3.13 ± 10.30), while MAF values did not differ significantly (p = 0.4), -3.9 ± 14.23 in cancer versus -1.05 ± 9.69 in normal mucosa (Figure 4). The highest MAF-values were detected among the healthy samples and the lowest MAF-values among the cancer samples.

The percentage of epithelial cells and viable cells, and also the heterogeneity and absolute calcein fluorescence values of cells were not significantly different between the control and tumor groups, meaning that the two groups were not different in their main characteristics. ANOVA analysis of tumor localization, left or right sided tumors, TNM stage, grade, age and gender or previous chemo-radiotherapy showed minor, not significant differences in MAF values of MDR1 and/or MRP1.

**Discussion**

Because of their high expression in normal gastrointestinal epithelium, MDR1 and MRP1 proteins were considered to be also highly active in colorectal cancers [12]. Early investigations showed higher mRNA and expression levels of MDR1 in colorectal cancers [24], and carcinogenesis [10,11,13], but immunocytochemical [14] and immunoblotting studies [15] have found decreased MDR1 expression in tumor cells compared to the maintained expression in normal mucosa. Furthermore, discrepancy was described between MDR1 mRNA levels and MDR-phenomenon, concluding that phosphorylation status and localisation of MDR1 showed the strongest correlation with functionality [16]. Some studies raised the
prognostic role of P-Gp in CRC [25,26], however recent studies found no impact of MDR-expression on survival [13,27,28], not even the largest study with 102 cases [29].

More studies found decreased MRP1 expression with no impact on survival in gastrointestinal tract carcinomas [30,31]. Significant association of elevated MRP2-expression (and not MDR1 or MRP1!) was found in cisplatin resistance [32]. Constitutive MRP1 expression was described in a study of primary and metastatic colorectal cancers, whereas the same study found elevated MRP1 expression in metastatic cases which underwent chemotherapy [33], underlining the impact of previous chemotherapy on the presence and function of these transporters in cancer cells.

The discrepancy between expression study results and clinical findings could be partly resolved with functional studies, which measure the direct transport activity of these pumps regardless of expression or any posttranslational modifications. On the other hand, there are other mechanisms possibly resulting in multidrug resistance phenomenon, so MDR1 and MRP1 proteins might not play key-role in colorectal malignancies [34-37]. Recent research of cancer stem cells (CSCs) in CRC brought the renaissance of the MDR-phenomenon, since the tumor repopulating side population of the resistant CSCs are expressing more ABC-transporters, especially ABCG2 (MXR, BCRP) [38-40].

The modified calcein assay for solid tumors is based on the calcein assay used for prognostication of leukaemia [2] with an added double viability and immunocytochemical staining for selecting living cancer cells. This method has never been used before to investigate large numbers of colorectal samples and up to now very few data is available on activity of these transporters either in healthy or in tumorous colon mucosa [41]. We determined the MDR1 and MRP1 functional activity of normal and cancerous enterocytes in 73 tumor and 11 normal mucosa samples, representing the largest functional study by now. According to our results, multidrug transporter activity of healthy colon mucosa is mainly covered by the functional activity of MDR1 protein, while MRP1 showed lower activity (Figure 4.). The significant MDR1 transporter activity in normal mucosa is in good correlation with previous findings that normal enterocytes express functioning MDR1 transporters. The significant lower mean MAF values detected in our colorectal cancer samples were mainly generated by the significant decrease in the mean functional activity of MDR1 transporter, while MAF-MRP1 was practically unchanged.

For now preoperative radio-chemotherapy represents a routine clinical practice in rectal cancers, which means neoadjuvant 50 Gy irradiation combined with 5-FU of the rectal cancers. This treatment had no significant effect on activity of MDR1 and/or MRP1 proteins in the rectal cases (n = 29) involved in our study. Not any other significant differences were found either between the various location of tumors or between left and right sided tumors.

The chemotherapy of colorectal cancer is based on 5-FU, which is neither MDR1 nor MRP1 substrate, but nowadays chemotherapeutic regimen is widening. Irinotecan (MDR1 substrate) and Oxaliplatin (MRP1 substrate) drugs were also involved and succeeded to increase the survival rate of patients. With these newer agents the MDR1 and MRP1 functional activity might influence the response to therapy and possibly also the survival of patients. Functional data determined with our modified calcein-assay protocol could provide more information and insight into the function of MDR-transporters in colorectal diseases. As clinical follow up is in progress, we will be able to study the impact of MDR1 and MRP1 functional activity on the survival of patient in several years.
Conclusion

In conclusion, our study is the first one to use the modified calcine-assay to determine the MDR1 and MRP1 functional activity of enterocytes and cancer cells from larger numbers of surgical samples of colorectal cancers and healthy mucosa. We measured the MAF<sub>Total</sub>, MAF<sub>MRP1</sub> and MAF<sub>MDR1</sub> values of 100 colorectal cancer and 28 normal mucosa samples of which 73 tumor and 11 normal mucosa gave sufficient cells for reliable statistical analysis. We found significant decrease in the MAF<sub>Total</sub> and MAF<sub>MDR1</sub> values of colorectal cancer cells compared to the adjacent, apparently normal mucosa. Normal mucosa showed significant MDR1 functional activity, but there was no detectable change in the low MRP1 functional activity between the normal and tumorous mucosa. Univariate and multivariate analysis of tumor localization, TNM stage, grade, age and gender showed no significant impact on multidrug functional activity. Our findings are in good correlation with previous expression studies of MDR1 and MRP1 proteins, which underlined that the expression of MDR1 protein in colorectal cancers is not primarily elevated and probably has no impact on survival of patients. Thus, contrary to general belief MDR activity seems not to play a major role in chemoresistance, but might rather explain preferential/selective activity of irinotecan and/or oxaliplatin in CRC. Still, this picture might be as simple, and it is unclear whether chemotherapy might alter this, and furthermore, today a very few is known about MDR activity in CSC. Although, the majority of the chemoresistance of primary CRCs might not be mediated through MDR1 or MRP1 proteins, the combination of predictive molecular diagnostics and MDR diagnostics can potentially further contribute to the advancement of personalized treatment of colorectal cancer patients.

Acknowledgements

We would like to thank the tremendous laboratory work to Beatrix B. Nemeth, Attila Varga, Eva Karaszi who performed most of the laboratory measurements in late hours after surgical resection.

Author details

1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Úllői út 26, H-1085 Budapest, Hungary. 2nd National Drug Design Laboratories, Cooperative Research Center, Semmelweis University, Úllői út 26, H-1085 Budapest, Hungary. 3rd Department of Surgery and Vascular Surgery, Uzsoki Teaching Hospital, Uzsoki street 29, H-1145 Budapest, Hungary. 4th Institute of Molecular Pathology, Department of Biological Nanochemistry, Pusztaszeri út 59-67, 1025 Budapest, Hungary. 5th Department of Surgery, Semmelweis University, Úllői út 78, 1082 Budapest, Hungary.

Received: 13 October 2014 Accepted: 7 April 2015

Published online: 16 April 2015

References

1. Ho GT, Moodie FM, Satsangi J. Multidrug resistance 1 gene (P-glycoprotein 170): an important determinant in gastrointestinal disease? Gut. 2003;52:759–66.
2. Karaszi E, Jakab K, Homolya L, Szakacs G, Hollo Z, Telek B, et al. Calcein assay for multidrug resistance reliably predicts therapy response and survival rate in acute myeloid leukemia. Br J Haematol. 2001;112:308–14.
3. Coley HM, Ventri MV, Gregson SE, Odell DE, Fisher C, Judson JR. Incidence of P-glycoprotein overexpression and multidrug resistance (MDR) reversal in adult soft tissue sarcoma. Eur J Cancer. 2000;36:881–8.
4. Chan HS, Thorner PS, Haddad G, Ling V. Immunohistochemical detection of P-glycoprotein: prognostic correlation in soft tissue sarcoma of childhood. J Clin Oncol. 1990;8:689–704.
5. Wang BL, Zhai HY, Chen BY, Zhai SP, Yang HY, Chen XP, et al. Clinical relationship between MDR1 gene and gallbladder cancer. Hepatobiliary Pancreat Dis Int. 2003;2:296–9.
6. Norris MD, Bordow SB, Marshall GM, Haber PS, Cohn SL, Haber M. Expression of the gene for multidrug-resistance-associated protein and outcome in patients with neuroblastoma. N Engl J Med. 1996;334:231–8.
7. Wang BL, Chen XP, Zhai SP, Chen DF. Clinical significance of mrp gene in primary hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int. 2003;2:397–403.
8. Oshika Y, Nakamura M, Tokunaga T, Fukushima Y, Abe Y, Ozeki Y, et al. Multidrug resistance-associated protein and mutant p53 protein expression in non-small cell lung cancer. Mod Pathol. 1998;11:1059–63.
9. Zimmermann C, Gutmann H, Hruz P, Gutzwiller JP, Beglinger C, Drewe J. Mapping of multidrug resistance gene 1 and multidrug resistance-associated protein isoform 1 to 5 mRNA expression along the human intestinal tract. Drug Metab Digox. 2005;33:219–24.
10. Peters WH, Boon CE, Roelofs HM, Wobbes T, Nagnestad FM, Kremers PG. Expression of drug-metabolizing enzymes and P-170 glycoprotein in colorectal carcinoma and normal mucosa. Gastroenterology. 1992;103:486–55.
11. Meijer GA, Schroeijers AB, Flens MJ, Meeuwen SG, van der Valk P, Baak JP, et al. Increased expression of multidrug resistance related proteins Pgp, MRP1, and LRP/MVP occurs early in colorectal carcinogenesis. J Clin Pathol. 1999;52:450–45.
12. Goldstein LJ, Galski H, Fojo A, Willingham M, Lai SL, Gazdar A, et al. Expression of a multidrug resistance gene in human cancers. J Natl Cancer Inst. 1998;91:116–24.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

TMI has performed measurements, statistical analysis, wrote manuscript. AL has performed measurements, wrote manuscript. TMe has participated in statistical analysis and acquiring surgical samples and writing manuscript, ZB participated in study design, surgical sampling, manuscript writing. IB Jr, KD, AZ, FJ have participated in surgical sampling, data interpretation and manuscript writing. LX has participated in statistical analysis, manuscript writing. GK participated in study design and manuscript writing. RS designed and performed study, participated in manuscript writing. IP has designed study, participated in data interpretation and manuscript writing. All authors have read and approved the final manuscript.
13. Pirker R, Wallner J, Guer A, Gotzl M, Zochbauer S, Scheithauer W, et al. 
MDR1 gene expression in primary colorectal carcinomas. Br J Cancer. 
1993;68:691–4.

14. Caruso ML, Valentini AM, Armentano R, Pirelli M. P-170 glycoprotein 
expression in gastric and colorectal carcinomas and normal mucosa. 
An immunocytochemical study. In Vivo. 1995;9:133–8.

15. Die Angélique P, Stokke T, Smedshammer L, Lothe RA, Lehne G, Chen Y, et al. 
P-glycoprotein is not expressed in a majority of colorectal carcinomas and 
is not regulated by mutant p53 in vivo. Br J Cancer. 1995;72:307–11.

16. Kramer R, Weber TK, Mors E, Arceci R, Staniunas R, Steele G Jr, et al. 
Constitutive expression of multidrug resistance in human colorectal 
tumours and cell lines. Br J Cancer. 1993;67:959–68.

17. Kramer R, Weber TK, Arceci R, Ramchuren N, Kastrinakis WJ, Steele G Jr, et al. Inhibition of N-linked glycosylation of P-glycoprotein by tunicamycin 
results in a reduced multidrug resistance phenotype. Br J Cancer. 
1995;71:670–5.

18. Lee WP. P-glycoprotein is hyperphosphorylated in multidrug resistant HOB1 
lymphoma cells treated with overdose of vincristine. Biochim Biophys Acta. 
1995;1245:57–61.

19. Zhang JT, Ling V. Study of membrane orientation and glycosylated 
extracellular loops of mouse P-glycoprotein by in vitro translation. 
J Biol Chem. 1991;266:18224–32.

20. Chua YJ, Cunningham D. Recent data with anti-epidermal growth factor 
receptor antibodies and irinotecan in colon cancer. Clin Colorectal Cancer. 
2005;5 Suppl 2:S81–8.

21. Wang WS, Chen PM, Su Y. Colorectal carcinoma: from tumorigenesis to 
treatment. Cell Mol Life Sci. 2006;63:663–71.

22. Schwab R, Micsik T, Szokoloczi O, Schafer E, Tihanyi B, Tihanyi T, et al. 
Functional evaluation of multidrug resistance transporter activity in surgical 
samples of solid tumors. Assay Drug Dev Technol. 2007;5:541–50.

23. Ruxton GD. The unequal variance t-test is an underused alternative to 
Student’s t-test and the Mann–Whitney U test. Behav Ecol. 2006;17:688–90.

24. Waqas O, Kamada K, Furuwaka T, Hidaka H, Hisaizu T, Shimazu H, et al. 
Expression of the MDR1 gene in human gastric and colorectal carcinomas. 
J Natl Cancer Inst. 1990;82:1679–83.

25. Sinicrope FA, Hart J, Brasitus TA, Michelle F, Lee JJ, Saha AR. Relationship of 
P-glycoprotein and carcinoembryonic antigen expression in human colon 
carcinoma to local invasion, DNA ploidy, and disease relapse. Cancer. 
1994;74:2008–17.

26. Weinstein RS, Jakate SM, Dominguez JM, Lembouitz MD, Koukoulis GK, 
Kuszak Jr, 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.

27. Tokunaga Y, Hosogi H, Hoppou T, Nakagami M, Tokuka A, Dharmi K. Effects 
of MDR1/P-glycoprotein expression on prognosis in advanced colorectal 
cancer after surgery. Oncol Rep. 2001;8:815–9.

28. Mayer A, Takimoto M, Fritz E, Schellander G, Kofler K, Ludwig H. The 
prognostic significance of proliferating cell nuclear antigen, epidermal 
growth factor receptor, and mdr gene expression in colorectal cancer. 
Cancer. 1993;71:2454–60.

29. Zochbauer S, Wallner J, Haider K, Depisch D, Huber H, Pirker R. MDR1 RNA 
transcripts do not indicate long-term prognosis in colorectal carcinomas. 
Eur J Cancer. 1997;33:1516–8.

30. Takebayashi Y, Akiyama S, Natsugoe S, Hokita S, Niwa K, Kitazono M, et al. 
Expression of multidrug resistance protein in human gastrointestinal 
tract carcinomas. Mol Biotechnol. 1999;14:308–16.

31. Fillipits M, Suchomel RW, Deakin G, Stigbauer W, Haider K, Depisch D, et al. 
Expression of the multidrug resistance-associated protein (MRP) gene in 
colorectal carcinomas. Br J Cancer. 1997;75:208–12.

32. Hinoshita E, Uchiyuma T, Taguchi K, Kinukawa N, Tsuneyoshi M, Maehara Y, et al. 
Increased expression of an ATP-binding cassette superfamily transporter, 
multidrug resistance protein 2, in human colorectal carcinomas. Clin Cancer 
Res. 2000;6:2401–7.

33. Nanashiama A, Yamaguchi H, Matsuo S, Sumida Y, Tsuchi T, Sawai T, et al. 
Expression of multidrug resistance protein in metastatic colorectal 
carcinomas. J Gastroenterol. 1999;34:582–8.

34. Gillet JP, Gottesman MM. Overcoming multidrug resistance in cancer: 
35 years after the discovery of ABCB1. Drug Resist Updat. 2012;15:2–4.

35. Baguley BC. Multiple drug resistance mechanisms in cancer. Mol Biotechnol. 
2010;46:308–16.