

RESEARCH COMMUNICATION

Expression of ABCG2 and its Significance in Colorectal Cancer

Hai Guang Liu¹, Yi Fei Pan^{1*}, Jie You¹, Ou Chen Wang¹, Ka Te Huang², Xiao Hua Zhang^{1*}

Abstract

The expression of ABCG2 in colorectal cancer and its relationship with invasion and metastasis is still not clear. In our study, immunohistochemical staining of ABCG2 was therefore performed for 60 cases of primary colorectal cancer. ABCG2 positive cancer cells were found to be mainly positioned in the front of carcinomatous tissue or between carcinomatous and non-carcinomatous margin tissues. In carcinomatous tissues and non-carcinomatous margin tissues, high expression rates for ABCG2 were 36.7% (22/60) and 3.3% (2/60) respectively, with significant difference ($\chi^2=5773.3$, $P<0.001$). The rates of high expression of ABCG2 were 30% (9/30) and 6.7% (2/30) in 30 cases with and without positive lymph nodes, respectively. ($\chi^2=5.45$, $P<0.025$). From the present results expression of ABCG2 may be important in the progression and metastasis of colorectal cancer.

Keywords: ABCG2 - colorectal cancer - metastasis

Asian Pacific J Cancer Prev, **11**, 845-848

Introduction

ABCG2 is a member of ATP binding cassette transporter family. It is identified by three different research groups and named breast cancer resistance protein (BCRP) (Doyle et al., 1998), ABCP for ABC transporter highly expressed in the placenta (Allikmets et al., 1998) and mitoxantrone resistance protein (MXR) (Miyake et al., 1999). ABCG2 is expressed in many normal tissues such as placenta, liver, small intestinal and colon, breast and amnion (Maliapaard et al., 2001; Aye et al., 2007). Besides that, ABCG2 is also expressed in many types of cancer such as multiple myeloma (Raaijmakers et al., 2005), advanced non-small cell lung cancer (Yoh et al., 2004), large B-cell lymphoma (Hu et al., 2008), colon cancer (Candeil et al., 2004). Many researches show that ABCG2 is associated with multi-drug resistance (Fletcher et al., 2010; Kalalinia et al., 2010; Kawahara et al., 2010; Pollex et al., 2010; Shigeta et al., 2010). Furthermore, ABCG2 is expressed in many types of stem cells and is a molecular determinant of the side-population phenotype (Zhou et al., 2001; Zheng et al., 2010). It may be a marker of cancer stem cells and a new target to cancer chemotherapy (Ding et al., 2009; Yan and Ongkeko, 2009). Therefore, ABCG2 is a research hot point in cancer chemotherapy.

Gupta et al (2006) quantified the expression of ABCG2 in paired normal and cancer cDNA samples from 154 patients with tumors in 19 different tissues. ABCG2 mRNA was present in normal colorectal tissue and showed a 6-fold decrease in cancer. The down-regulation of ABCG2 mRNA and protein was also evident in cervical cancer. There was also a decrease in ABCG2 mRNA in

cancer in 12 of the 19 different tissues collected from the 154 patients, indicating that cancer-associated down-regulation of ABCG2 was associated with various cancer types likely to be a common phenomenon in several tissues. However, Zen et al (2007) reported that there was an increase in ABCG2 mRNA and protein in three HCC cell lines and seven cases of human hepatocellular carcinoma, compared to normal liver samples (n=2). Therefore, the expression of ABCG2 in cancer is not well defined.

In this study, we conducted histological immunostaining of colorectal cancer specimens to evaluate the expression of ABCG2 and its significance.

Materials and Methods

Immunohistochemistry and immunofluorescence

Tissue samples obtained for immunohistochemistry were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections (4 μ m) cut from the paraffin block were deparaffinized in xylene and rehydrated through graded alcohols. Normal, cancer and lymph node tissues from the same patient were mounted on the same slide to ensure identical conditions. The multi-clonal anti-ABCG2 antibody was used as the primary antibody at a dilution of 1:100. The secondary antibody was goat-anti-mouse IgG. Negative controls were exposed to PBS in place of primary antibody, and were processed in the same manner. For immunofluorescence, the secondary antibody was replaced with FITC-goat-anti-mouse IgG, and incubated with 2 μ g per ml propidium iodide (Sigma) for 30 minutes. Sections were viewed using an Olympus confocal microscope

¹Department of Oncology, the First Affiliated Hospital, Wenzhou Medical College, ²Department of Pathology, the First Affiliated Hospital, Wenzhou Medical College, China. * For correspondence : zhangxhwzmc@yahoo.com, panyfwzmc@yahoo.com

equipped with a Spot Camera and Spot Software.

Evaluation standards for immunohistochemistry

Both the extent and intensity of immunopositivity were considered when scoring ABCG2 expression. The extent of positivity was scored as follows: 0, <5%; 1, 5-25%; 2, 25-50%; 3, 50-75%; and 4, >75% of cancer cells in the respective lesions. The intensity was scored as follows: 0, negative; 1+, weak; 2+, moderate; and 3+, strong. The final scores were obtained by multiplying the extent of positivity and intensity scores, producing a range from 0 to 12. Scores 9-12 were defined as a preserved or strong staining pattern, scores 0-4 were defined as markedly reduced or lost expression, and scores 5-8 were defined as an intermediate staining pattern (Hao et al., 2000; Hoa et al., 2001). Scores 5-12 were defined as high expression and scores 0-4 were defined as low expression. The results were evaluated by two pathologists blinded to the identity of the samples.

Statistical analysis

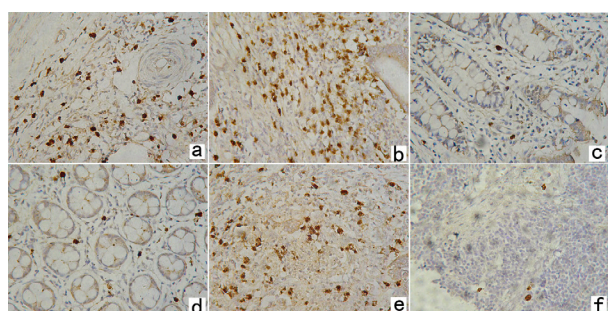


Figure 1. ABCG2 Immunohistochemistry in Colorectal Cancer a, b: Cancer tissues ×400; c, d: Normal margins of surgery ×400; e: Positive lymph nodes ×400; f: Negative lymph nodes ×400; ABCG2 was yellow or brown-yellow following DAB staining under an optical microscope. ABCG2 was highly expressed in cancer tissues (a, b) and positive lymph nodes (e), and had decreased expression in normal margins of surgery (c, d) and negative lymph nodes (f).

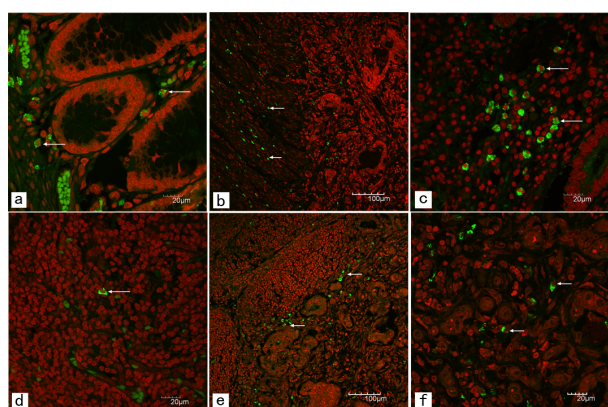


Figure 2. ABCG2 Immunofluorescence in Colorectal Cancer a: Normal margins of surgery; b, c: Cancer tissues; d: Negative lymph nodes; e, f: Positive lymph nodes; After staining with FITC-goat anti-mouse IgG and PI, ABCG2 was stained green and the nucleoli were red under confocal microscopy. ABCG2 was mainly expressed in the membranes of cells (arrowhead marking). It was highly expressed in cancer tissues (b, c) and positive lymph nodes (e, f), and showed decreased expression in normal margins of surgery (a) and negative lymph nodes (d).

All statistical analyses were performed using the SPSS package (Windows, v.11). The Pearson Chi-Square tests was used to compare the ABCG2 expression between colorectal cancer tissues and non-carcinomatous tissues, and between positive lymph nodes and negative lymph nodes.

Results

ABCG2 expression in colorectal cancer tissues

After being stained with DAB developer, ABCG2 positive staining (indicated by yellow or brown-yellow under an optical microscope) was mainly found in membranes (Figure 1). In the 60 cases of carcinomatous tissues and non-carcinomatous margin tissues, the high expression rates of ABCG2 were 36.7% (22/60) and 3.3% (2/60), respectively. This was a significant difference between the expression in the malignant versus non-malignant tissues ($\chi^2=5773.3$, $P<0.001$) (Table 1). The rates of high expression of ABCG2 were 30% (9/30) and 6.7% (2/30) in 30 cases with positive lymph nodes and 30 cases with negative lymph nodes respectively. This was also significant difference between the two groups ($\chi^2=5.45$, $P<0.025$) (Table 2), indicating that both the presence of the primary tumor and invasive disease correlated with ABCG2 expression.

Confirmation of ABCG2 expression in colorectal cancer tissues by immunofluorescence

After staining with FITC-goat-anti-mouse IgG and PI, ABCG2 was observed to be green, while the cell nucleoli was red under confocal microscopy. The ABCG2 expression levels and frequency were similar to the immunohistochemistry results, confirming that ABCG2 is more highly expressed in carcinomatous tissue compared to normal tissues, and more in invasive disease than in localized disease (Figure 2). Furthermore, ABCG2 positive cancer cells were mainly positioned in the front of carcinomatous tissue or between carcinomatous and non-carcinomatous margin tissues (Figure 2 b and e). This phenomenon demonstrated that ABCG2 positive cancer

Table 1. Comparison of ABCG2 Expression between Colorectal Cancer Tissues and Non-carcinomatous Tissues

Groups	n	ABCG2		χ^2	P value ^a
		High expression	Low expression		
Cancer tissues	60	38	22	5773.3	<0.001
Non-carcinomatous tissues	60	2	58		

a: Pearson Chi-Square test.

Table 2. Comparison of ABCG2 Expression between Positive Lymph Nodes and Negative Lymph Nodes

Groups	n	ABCG2		χ^2	P value ^a
		High expression	Low expression		
Positive lymph nodes	30	9	21	5.45	<0.0025
Negative lymph nodes	30	2	28		

a: Pearson Chi-Square test.

cells might be associated with local invasion.

Discussion

ABCG2 is a member of ATP binding cassette transporter family and is associated with resistance to cancer chemotherapy. However, its expression in colorectal cancer and its relationship with invasion and metastasis is still not clear. Our data showed that In the 60 samples of carcinomatous and non-carcinomatous margin tissues, ABCG2 was highly expressed in 36.7% (22/60) and 3.3% (2/60) of the tissues, respectively, and in 30% (9/30) and 6.7% (2/30) of the 30 cases with positive lymph nodes and 30 cases with negative lymph nodes, respectively. Thus, ABCG2 is highly expressed in colorectal cancer, and is also more highly expressed by invasive cancers compared to those with no lymph node involvement. This suggests that ABCG2 may be involved in cancer progression and metastasis. This is consistent with Haraguchi's (2006) and Zen's (2007) reports. Gupta et al (2006) reported that ABCG2 mRNA was present in normal colorectal tissue but showed a 6-fold decrease in cancer. They suggested that decreased expression of ABCG2 might have a role in carcinogenesis by allowing the accumulation of genotoxins and over-production of nitric oxide, and that down-regulation of ABCG2 was likely to be a common phenomenon in several tissues.

We presumed that the expression of ABCG2 might be different in different stages of carcinogenesis. In the early stage of carcinogenesis, ABCG2 might be down-regulated to allow accumulation of genotoxins and over-production of nitric oxide. However, in the more advanced stages of carcinogenesis, it might be up-regulated to expel chemotherapeutic drugs to protect cancer cells. Therefore, we hypothesized that cancer-associated down-regulation of ABCG2 was not likely to be a common phenomenon, and that the expression of ABCG2 would be associated with more advanced cancers.

In conclusion, our study demonstrated that ABCG2 was highly expressed in colorectal cancer and positive lymph nodes, suggesting up-regulation in advanced malignancy, and ABCG2 positive cells were mainly positioned in the front of carcinomatous tissue or between carcinomatous and non-carcinomatous margin tissues. Therefore, we believe that ABCG2 is important in the progression and metastasis of colorectal cancer and that it may be a new target of cancer therapy.

Acknowledgements

We acknowledge Wenzhou Science and Technology Project Y200090008 to support this research. The authors declare that they have no competing interests with regard to the study.

References

Allikmets R, Schriml LM, Hutchinson A, et al (1998). A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. *Cancer Res*, **58**, 5337-9.

Expression of ABCG2 and its Significance in Colorectal Cancer

- An Y, Ongkeko WM (2009). ABCG2: The key to chemoresistance in cancer stem cells? *Expert Opin Drug Metab Toxicol*, **5**, 1529-42.
- Aye IL, Paxton JW, Evseenko DA, et al (2007). Expression, localisation and activity of ATP binding cassette (ABC) family of drug transporters in human amnion membranes. *Placenta*, **28**, 868-7.
- Candeil L, Gourdiere I, Peyron D, et al (2004). ABCG2 overexpression in colon cancer cells resistant to SN38 and in irinotecan-treated metastases. *Int J Cancer*, **109**, 848-54.
- Ding XW, Wu JH, Jiang CP (2010). ABCG2: A potential marker of stem cells and novel target in stem cell and cancer therapy. *Life Sci*, **86**, 631-7.
- Doyle LA, Yang W, Abruzzo LV, et al (1998). A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc Natl Acad Sci U S A*, **95**, 15665-70.
- Fletcher JI, Haber M, Henderson MJ, et al (2010). ABC transporters in cancer: more than just drug efflux pumps. *Nat Rev Cancer*, **10**, 147-56.
- Gupta N, Martin PM, Miyauchi Si, et al (2006). Down-regulation of BCRP/ABCG2 in colorectal and cervical cancer. *Biochem Biophys Res Commun*, **343**, 571-7.
- Haraguchi N, Utsunomiya T, Inoue H, et al (2006). Characterization of a side population of cancer cells from human gastrointestinal system. *Stem Cells*, **24**, 506-13.
- Hao XP, Willis JE, Pretlow TG, et al (2000). Loss of fragile histidine triad expression in colorectal carcinomas and premalignant lesions. *Cancer Res*, **60**, 18-21.
- Hao XP, Pretlow TG, Rao JS, et al (2001). Beta-catenin expression is altered in human colonic aberrant crypt foci. *Cancer Res*, **61**, 8085-8.
- Hu LL, Wang XX, Chen X, et al (2007). BCRP gene polymorphisms are associated with susceptibility and survival of diffuse large B-cell lymphoma. *Carcinogenesis*, **28**, 1740-4.
- Kalalinia F, Elahian F, Behravan J (2010). Potential role of cyclooxygenase-2 on the regulation of the drug efflux transporter ABCG2 in breast cancer cell lines. *J Cancer Res Clin Oncol*, **27**.
- Kawahara H, Noguchi K, Katayama K, et al (2010). Pharmacological interaction with sunitinib is abolished by a germ-line mutation (1291T>C) of BCRP/ABCG2 gene. *Cancer Sci*, **101**, 1493-500.
- Maliapaard M, Scheffer GL, Faneyte IF, et al (2001). Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. *Cancer Res*, **61**, 3458-64.
- Miyake K, Mickley L, Litman T, et al (1999). Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. *Cancer Res*, **59**, 8-13.
- Pollex EK, Anger G, Hutson J, et al (2010). Breast cancer resistance protein (BCRP)-mediated glyburide transport: Effect of the C421A/Q141K BCRP single-nucleotide polymorphism. *Drug Metab Dispos*, **38**, 740-4.
- Raaijmakers MH, de Grouw EP, Heuver LH, et al (2005). Impaired breast cancer resistance protein mediated drug transport in plasma cells in multiple myeloma. *Leuk Res*, **29**, 1455-8.
- Shigeta J, Katayama K, Mitsuhashi J, et al (2010). BCRP/ABCG2 confers anticancer drug resistance without covalent dimerization. *Cancer Sci*, **101**, 1813-21.
- Yoh K, Ishii G, Yokose T, et al (2004). Breast cancer resistance protein impacts clinical outcome in platinum-based chemotherapy for advanced non-small cell lung cancer. *Clin Cancer Res*, **10**, 1691-7.
- Zen Y, Fujii T, Yoshikawa S, et al (2007). Histological and

culture studies with respect to ABCG2 expression support the existence of a cancer cell hierarchy in human hepatocellular carcinoma. *Am J Pathol*, **170**, 1750-62.

Zhou S, Schuetz JD, Bunting KD, et al (2001). The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat Med*, **7**, 1028-34.

Zheng X, Cui D, Xu S, Brabant G, et al (2010). Doxorubicin fails to eradicate cancer stem cells derived from anaplastic thyroid carcinoma cells: characterization of resistant cells. *Int J Oncol*, **37**, 307-15.