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学位論文題目	ABCG2 expression in colorectal adenocarcinomas may predict resistance to irinotecan. （大腸癌における ABCG2 の発現はイリノテカンへの抵抗性を予測する）
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論 文 内 容 要 旨

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学位論文題目	**ABCG2 EXPRESSION IN COLORECTAL ADENOCARCINOMAS MAY PREDICT RESISTANCE TO IRINOTECAN** (大腸癌における ABCG2 の発現はイリノテカンへの抵抗性を予測する)		

INTRODUCTION

Irinotecan is a key drug for patients with advanced and recurrent colorectal carcinoma. However, its efficacy is not sufficient, partly because there is no useful marker that predicts chemosensitivity to this drug. ATP-binding cassette sub-family G member 2 (ABCG2) is major types of multidrug resistance which is the principal reason for failure of anticancer chemotherapy. Overexpression of ABCG2 protein in colon cancer cell lines is involved in high levels of resistance to SN38 in vitro.

The aim of this study was to assess whether immunohistochemical expression of ABCG2 can be a potential predictor of the response to irinotecan-based treatment of colorectal cancer patients.

MATERIALS AND METHODS

Using resected primary tumor specimens of 189 colorectal cancer patients, we investigated the correlation between immunohistochemical expression of ABCG2 protein and results of the collagen gel droplet embedded culture test (CD-DST) of chemosensitivity to SN38, an active metabolite of irinotecan.

The staining intensity of positive cell membranes was classified as negative (0, no staining), weak (1), moderate (2), or intense (3, as strong as in normal colonocytes) and the proportion of total positive cancer cells with membranous positivity was scored as follows: 0 (< 5%), 1 (5–25%), 2 (26–50%), 3 (51–75%) or 4 (> 75%). ABCG2 expression was determined by multiplication of the values for intensity and proportion, and was classified as low-expression or high-expression for scores of 0-8 or 9-12.

CD-DST was used to evaluate the sensitivity of cancer tissue to SN38. Briefly, colorectal cancer specimens obtained by surgery were digested with collagenase, and the dispersed cancer cells were incubated in a collagen gel-coated flask. Only the viable cells adhering to the collagen gel layer were collected and added to reconstructed type I collagen solution Anticancer agents were added to each well for 24 hours. After that each well was incubated with PCM-2 medium for 7 days. The in vitro chemosensitivity effect was expressed as a ratio of the total colony volume of the treated group (T) to that of the control group (C) (T/C ratio). A T/C ratio of 60% or less was regarded as sensitive.

Among 189 patients, 17 had irinotecan-based chemotherapy. Their responses and progression-free survival (PFS) were analyzed.

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等で印字すること。

２．※印の欄には記入しないこと。

RESULTS

Expression of ABCG2 in colorectal cancer tissues

The patients were classified into the low-expression group (76 patients, 40%) and the high-expression group (113 patients, 60%), of which the *median immunohistochemistry scores* were 4.21 ± 0.25 and 10.55 ± 0.15, respectively.

Correlation with SN38 response of ABCG2 expression and clinicopathological factors in colorectal cancer patients

Among 189 patients, 119 patients had colon cancer and 70 rectal cancers. Patients' ages ranged from 33 to 88 years (median 65). Moderate differentiation (72%) and stage 3 or 4 (54%) were found in the majority. The median SN38 T/C ratio was significantly higher in the high-expression group than the low-expression group ($p < 0.001$).

Patients with high expression of ABCG2 were significantly more resistant to SN38 ($p < 0.001$). The sensitivity of high expression of ABCG2 to predict the low response to SN38 by CD-DST, was 82%, and the specificity was 73%.

Patients with high expression of ABCG2 were strongest indicator of resistant to SN38 by multivariate analysis (PR: 11.77, 95%CI: 5.83-23.76, $p<0.001$).

ABCG2 expression and clinical response to irinotecan-based chemotherapy

High expression of ABCG2 was found in 11 of 12 non-responders, whereas 4 of 5 responders had low expression. The sensitivity of high ABCG2 expression to predict the resistance to irinotecan-based chemotherapy was 92%, and the specificity was 80%. High expression of ABCG2 and low sensitivity to SN38 significantly correlated with the resistance to irinotecan-based chemotherapy ($p = 0.01$ and 0.028, respectively)

The median PFS of the responder group was significantly longer than the non-responder group (372 vs. 104 days, $p = 0.013$). The median PFS of patients with high expression of ABCG2 was significantly shorter than those with low expression (104 vs. 242 days, $p = 0.047$). The median OS of the patients with low and high ABCG2 expression were 449 days and 554 days, respectively, without significant difference ($p = 0.505$).

DISCUSSION

Gupta et al. reported that expression of ABCG2 mRNA and protein was abundant in the normal colon and decreased in colon cancer tissue. However, a possibility of the alterations in ABCG2 expression during progression in carcinoma remained to be clarified. This study demonstrated that around 60% of the colon carcinoma showmed high expression of ABCG2. Immunohistochemistry staining of ABCG2 in primary colorectal cancer tissues can be performed easily compared with CD-DST because it doesn't need fresh specimen. In the present study, despite having only 17 eligible patients, high expression of ABCG2 was significantly associated with resistance to irinotecan-based chemotherapy ($p = 0.01$) and shorter PFS ($p = 0.047$). These data suggest that expression of ABCG2 might be useful as a biomarker to predict chemo-resistance to irinotecan-based chemotherapy of colorectal cancer patients.

Prospective studies with larger numbers of patients are needed to confirm this hypothesis. Possibly, an inhibitor of ABCG2 could be useful to increase the chemo-sensitivity to irinotecan-based regimens in patients with colon cancer patients.

CONCLUSION

High expression of ABCG2 could be involved in SN38 resistance in colorectal cancer and might be a useful predictive biomarker for use in these patients, who may be under consideration for treatment with irinotecan-based chemotherapy.

別紙様式８（課程・論文博士共用）

学位論文審査の結果の要旨

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（学位論文審査の結果の要旨）

発表者らは、計 189 例の大腸癌患者の切除標本を用いて、癌組織の免疫染色における ABCG2（ATP-binding cassette sub-family G member 2）の発現度と CD-DST を用いた *in vitro* での SN38（ｲﾘﾉﾃｶﾝ代謝活性物）抵抗性との関係性や、計 17 例のｲﾘﾉﾃｶﾝを含む化学療法施行例における ABCG2 の発現度と PFS（progression-free survival；無増悪生存期間）との関係性について検討を行い、以下の点を明らかにした。

１）全症例において、ABCG2 高発現度と SN38 抵抗性の感度は 82%、特異度は 73%であった。
２）多変量解析において、ABCG2 高発現度は SN38 抵抗性と高い相関を示した（PR:11.77, 95%CI 5.83-23.76, p<0.001）。
３）化学療法施行例において、ABCG2 高発現度とｲﾘﾉﾃｶﾝ抵抗性の感度は 92%、特異度は 80%であった。
４）ABCG2 低発現群と比較して、ABCG2 高発現群の PFS 中央値は優位に低値であった（p<0.047）。

本論文は、大腸癌における ABCG2 発現度がｲﾘﾉﾃｶﾝ抵抗性を予測するﾊﾞｲｵﾏｰｶｰとなりうる可能性について新しい知見を与えたものであり、最終試験として論文内容に関連した試問を受け合格したので、博士（医学）の学位論文に値するものと認められた。

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