# Expression and Prognostic Significance of NPC1L1 in Colorectal Cancer: A Retrospective Cohort Study

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**Research Article**

**Keywords:** NPC1L1, ezetimibe, cholesterol, colorectal cancer, prognosis

**Posted Date:** June 16th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-614918/v1

# Abstract

**Background**: Colorectal cancer (CRC) is a malignant tumor of the large intestine. Studies have shown that the development and prognosis of CRC are associated with altered lipid metabolism. Niemann-Pick C1-Like 1 (NPC1L1), the target of ezetimibe, plays an essential role in the absorption of intestinal cholesterol. However, the role of altered NPC1L1 expression in the development and prognosis of CRC has not yet been determined.

**Methods**: Datasets of patients with CRC were obtained from The Cancer Genome Atlas (TCGA) database. To compare the expression of NPC1L1 in normal and CRC tissues, datasets obtained from the GDAC platform were used. To support these results, we also analyzed other datasets from the Gene Expression Omnibus (GEO) database. Student's *t*-test and chi-square test were used for the analyses. The log-rank test and multivariate Cox proportional hazards regression analysis were performed to determine whether NPC1L1 is a significant factor affecting the prognosis of CRC.

**Results**: The mRNA expression of NPC1L1 was found to be upregulated in CRC, and was significantly associated with the N- and pathological stages, but not with the histological type, age, and sex. Moreover, an increase in NPC1L1 expression in CRC was associated with poorer survival, based on the Kaplan–Meier and multivariate regression analyses.

**Conclusions**: High expression of NPC1L1 is associated with CRC development, pathological stage, and prognosis. The present study suggests that NPC1L1 represents a potential independent prognostic marker for CRC.


# Background

Colorectal cancer (CRC), a malignancy of the colon and rectum, is the third most common tumor and the fourth leading cause of cancer-related deaths globally [1]. The incidence of CRC is rapidly increasing in medium-to-high human development index (HDI) countries such as those in Eastern Europe, South America, and Asia due to changes in dietary habits and westernized lifestyles [2, 3]. The incidence rate of CRC is 19.7/100,000 people, and the 5-year survival rate of patients with stage IV or metastatic CRC is a dismal 12% [4]. Age, family history, inflammatory bowel disease (IBD), hereditary CRC, obesity, and diabetes are known risk factors for CRC. However, despite advances in our understanding of CRC, advanced CRC responds poorly to conventional chemotherapy, and often has a poor prognosis. Therefore, it is necessary to identify new molecular markers for the prediction and estimating the prognosis of CRC, and to improve the survival rates of CRC patients.

Recently, many studies have reported that the development and progression of cancers are associated with altered cholesterol metabolism [5]. Oxysterol 27-hydroxycholesterol, a primary metabolite of cholesterol, promotes breast cancer progression in mouse models [6], and the level of total cholesterol is positively associated with the incidence of prostate cancer in men [7]. High levels of cholesterol intake have been associated with an increased risk of CRC [8], and elevated serum cholesterol is correlated with

CRC risk [9]. Among the molecules involved in cholesterol metabolism, Niemann-Pick C1-Like 1 (NPC1L1) is a transmembrane protein that is essential for intestinal cholesterol absorption [10]. NPC1L1 is a target for ezetimibe, which is used to treat dyslipidemia that is not controlled by statins. It is highly expressed in the human small intestine and liver and is expressed at low levels in the human colon, kidney, and brain [11]. NPC1L1 knockdown was reported to significantly decrease the number of tumors formed in a murine model of colitis-associated CRC [12]. However, whether altered NPC1L1 expression is correlated with the development and prognosis of CRC in humans remains unclear.

In the present study, we compared NPC1L1 expression in non-tumor tissues and CRCs and examined the influence of NPC1L1 on the overall survival (OS) of patients with CRC. Moreover, we performed a correlation analysis between the expression of NPC1L1 and clinical characteristics and determined whether NPC1L1 is an independent prognostic factor for CRC.

## Materials And Methods

# Patients and Gene expression data

The Cancer Genome Atlas (TCGA) database provides large clinical datasets with information on DNA methylation, microRNAs, and RNA expression in various types of cancers, including CRC. We obtained datasets of patients with CRC from the TCGA database, as previously described [13]. In this study, datasets of 629 patients with CRC were obtained from the TCGA database. However, 201 patients were excluded from the study owing to the unavailability of gene expression data and clinical information. Thus, a total of 428 patients with CRC were finally evaluated in this study (Fig. 1).

To compare the expression of NPC1L1 in non-tumor and CRC tissues, datasets for NPC1L1 expression in normal tissues paired with CRC were obtained from the GDAC platform (http://gdac.broadinstitute.org/). GDAC is a database that stores the genomic data of cancer patients for TCGA projects and is open for research purposes. To confirm the expression and prognostic significance of NPC1L1 in CRC, we obtained and analyzed other datasets (GSE9348 and GSE17536) from the Gene Expression Omnibus (GEO) database. The GEO platform (https://www.ncbi.nlm.nih.gov/geo/) stores data such as those pertaining to microarrays and next-generation sequencing, and is operated by the National Center for Biotechnology Information (NCBI).

# Statistical analysis

The differences between the expression of NPC1L1 in non-tumor and CRC tissues were examined using the Student's *t*-test following the application of the Shapiro–Wilk normality test. Statistical significance was set at $P < 0.05$. A chi-squared test was performed to analyze the correlation between NPC1L1 expression and clinical characteristics. The OS was calculated from the date of diagnosis to death or last follow-up. For the Kaplan-Meier curves, patients with CRC were assigned to two groups: low (< median, n = 214) and high (> median, n = 214) expression groups based on the median levels of NPC1L1 expression. The log-rank test was used to calculate the P values. Univariate and multivariate Cox proportional

hazards regression analyses were conducted to determine whether NPC1L1 is a significant diagnostic marker of OS in patients with CRC.

## Results

# Baseline characteristics

To determine whether NPC1L1 expression is associated with CRC prognosis, we initially summarized the characteristics of the patients with CRC (Table 1). The mean age and OS (in months) of patients with CRC were 66.63 ± 13.08 and 29.43 ± 26.08, respectively. The type of CRC was mostly adenocarcinoma (n = 363, 84.8%), and the male-to-female ratio did not different notably (males: n = 228, females: n = 200). The percentage of CRC in white subjects (n = 202, 47.2%) was relatively high, and the percentage of Asian subjects with CRC was 2.6%. According to the American Joint Committee on Cancer (AJCC) staging system, the percentages of patients with stages I, II, III, and IV of CRC were 16.8%, 39.5%, 29.4%, and 14.3%, respectively.

Table 1
Clinical characteristics of patients with CRC

| Patient characteristics (n = 428) | | Total (%) |
|---|---|---|
| Histological type | Colon Adenocarcinoma | 363 (84.8) |
| | Colon Mucinous Adenocarcinoma | 60 (14) |
| | Unknown | 5 (1.2) |
| Overall survival months (mean ± SD*) | | 29.43 ± 26.08 |
| Age (mean ± SD*) | | 66.63 ± 13.08 |
| Sex | Male | 228 (53.3) |
| | Female | 200 (46.7) |
| Race | American Indian or Alaska native | 1 (0.2) |
| | Asian | 11 (2.6) |
| | Black or American | 57 (13.3) |
| | White | 202 (47.2) |
| | Unknown | 157 (36.7) |
| T stage | Tis | 1 (0.2) |
| | T1–T2 | 81 (18.9) |
| | T3–T4 | 346 (80.8) |
| M stage | M0 | 321 (75) |
| | M1 | 61 (14.3) |
| | Unknown | 46 (10.7) |
| N stage | N0 | 250 (58.4) |
| | N1–N2 | 178 (41.6) |
| AJCC stage | Stage I | 72 (16.8) |
| | Stage II | 169 (39.5) |
| | Stage III | 126 (29.4) |
| | Stage IV | 61 (14.3) |
| * SD: standard deviation | | |

# Expression of NPC1L1 in CRC

To compare NPC1L1 expression in normal and CRC tissues, RNA expression values of NPC1L1 obtained from the TCGA were represented in the box and whisker plot format. NPC1L1 expression was significantly higher in CRC tissues than in normal tissues (normal: mean 7.00, CRC: mean 130.09, $P < 0.05$) (Fig. 2A). For further confirmation, the relative expression of NPC1L1 was analyzed in non-tumor and tumor tissues from the same patients with CRC (Fig. 2B). The values of NPC1L1 expression in CRC tissues were individually divided by those for the paired normal tissues and presented as a bar graph. NPC1L1 expression was higher in most CRC tissues than in paired normal tissues. To determine whether the NPC1L1 expression levels in normal and tumor tissues from other data are consistent with the results obtained from the TCGA data, we also analyzed a dataset (GSE9348) from the GEO database (Fig. 2C). The expression of NPC1L1 was significantly higher in CRC tissues than in normal tissues (normal: mean 22.69, CRC: mean 81.35, $P < 0.05$). These results showed that high expression of NPC1L1 may be associated with CRC development.

# Relationship between NPC1L1 expression and clinical characteristics

Next, based on the median value of NPC1L1 expression, we divided the samples into the low and high NPC1L1 expression groups, and then examined the correlation between the expression of NPC1L1 and clinical characteristics of patients with CRC (Table 2). The expression level of NPC1L1 was significantly correlated with the N ($P < 0.05$) and pathological stages ($P < 0.05$). However, the expression level of NPC1L1 was not correlated with histological type, age, sex, and the T and M stages. These results indicate that high NPC1L1 expression may be associated with an advanced stage of CRC.

Table 2
Correlation between NPC1L1 expression and clinical characteristics of patients with CRC

| NPC1L1 expression | | | | |
|---|---|---|---|---|
| Characteristic | N | Low | High | P-value |
| Histologic type | 423 | | | P = 0.257 |
| Adenocarcinoma | | 186 | 177 | |
| Mucinous | | 26 | 34 | |
| Age (years) | 428 | | | P = 0.923 |
| < 70 | | 115 | 116 | |
| ≥ 70 | | 99 | 98 | |
| Sex | 428 | | | P = 0.245 |
| Male | | 120 | 108 | |
| Female | | 94 | 106 | |
| T stages | 428 | | | P = 0.141 |
| Tis, T1-T2 | | 47 | 35 | |
| T3-T4 | | 167 | 179 | |
| M stages | 382 | | | P = 0.069 |
| M0 | | 167 | 154 | |
| M1 | | 24 | 37 | |
| N stages | 428 | | | P = 0.006 |
| N0 | | 139 | 111 | |
| N1-N2 | | 75 | 103 | |
| AJCC stages | 428 | | | P = 0.025 |
| Stage I-II | | 132 | 109 | |
| Stage III-IV | | 82 | 105 | |

# Association between NPC1L1 expression and prognosis in CRC

To determine whether NPC1L1 expression affects the prognosis of patients with CRC, Kaplan-Meier survival analysis was performed for low and high NPC1L1 expression against OS (low expression group, n = 214; high expression group, n = 214) (Fig. 3A). Based on the 75% percentile, the OS of the low NPC1L1

expression group was 49.38 months whereas that of the high NPC1L1 expression group was 28.19 months. Thus, the OS of CRC patients with high NPC1L1 expression was significantly worse (P = 0.021). To confirm this result, another cohort (GSE17536) of patients with CRC was analyzed (low expression group, n = 148; high expression group, n = 29). Consistent with the results of the TCGA cohort, patients with NPC1L1-overexpressing CRC had significantly poorer survival (P = 0.036) (Fig. 3B). To further assess the prognostic significance of NPC1L1 expression, univariate and multivariate Cox proportional hazard regression analyses were performed (Table 3). In the univariate analysis, age (P = 0.015), AJCC stage (P < 0.001), and NPC1L1 (P = 0.022) were found to be indicators of OS. In multivariate analysis, age (P < 0.001), AJCC stage (P < 0.001), and NPC1L1 (P = 0.004) were also significantly associated with OS. The hazard ratios for age, stage, and NPC1L1 were 2.286, 3.698, and 1.618, respectively. Moreover, we compared the prognostic importance of NPC1L1 with that of other prognostic markers (VEGF-A, vascular endothelial growth factor A; MACC1, metastasis-associated in colon cancer 1; TGFB1, transforming growth factor-beta 1) in CRC (Table 4). Multivariate regression analysis showed that NPC1L1 (P = 0.038), together with VEGF-A (P = 0.009), was significantly associated with the OS of patients with CRC. These results indicate that NPC1L1 expression represents an independent marker for survival in CRC.

Table 3
Univariate and multivariate analysis of prognostic factors for OS in patients with CRC

| | **Univariate analysis** | **Multivariate analysis** | | | |
|---|---|---|---|---|---|
| | | **P-value** | **Hazard Ratio** | **CI* (lower 95%)** | **CI* (upper 95%)** |
| Age < 70 (vs ≥ 70) | P = 0.015 | P < 0.001 | 2.286 | 1.485 | 3.519 |
| Sex Male (vs Female) | P = 0.513 | P = 0.776 | 0.942 | 0.622 | 1.426 |
| Stage I + II (vs III + IV) | P < 0.001 | P < 0.001 | 3.698 | 2.366 | 5.779 |
| NPC1L1 Low (vs High) | P = 0.022 | P = 0.024 | 1.618 | 1.067 | 2.453 |
| * CI: confidence interval | | | | | |

Table 4
Univariate and multivariate analysis of NPC1L1 expression with other prognostic factors of CRC

| | Univariate analysis | Multivariate analysis | | | |
|---|---|---|---|---|---|
| | | p-value | Hazard Ratio | CI *(lower 95%) | CI *(upper 95%) |
| NPC1L1 Low (vs High) | P = 0.021 | P = 0.038 | 1.557 | 1.025 | 2.363 |
| VEGF-A Low (vs High) | P = 0.005 | P = 0.009 | 1.758 | 1.150 | 2.686 |
| MACC1 Low (vs High) | P = 0.033 | P = 0.106 | 1.421 | 0.928 | 2.178 |
| TGFB1 Low (vs High) | P = 0.132 | P = 0.056 | 1.502 | 0.990 | 2.278 |
| * CI: confidence interval | | | | | |

# Discussion

NPC1L1 is a known target of ezetimibe, which is used to treat dyslipidemia. However, its prognostic relevance in cancers has been poorly characterized. This study showed, for the first time, that NPC1L1 is upregulated in CRC and is associated with N and pathological stages. Moreover, the present study showed that high NPC1L1 expression in CRC is associated with poorer survival, suggesting that NPC1L1 is an independent prognostic marker in CRC.

Chen et al. showed that NPC1L1 was expressed at lower levels in hepatocellular carcinoma (HCC) compared to that in liver tissues adjacent to HCC tissues [14]. However, in the present study, NPC1L1 expression was increased in CRC compared to that in normal tissues (Fig. 2). Thus, the results suggest that NPC1L1 may be involved in the development of cancer and that NPC1L1 expression in cancer is type-specific.

A previous study showed that NPC1L1 expression was inversely correlated with the pathological stage, tumor differentiation, and vascular invasion in HCC [14]. In contrast, in the present study, NPC1L1 expression was positively associated with the N and pathological stages (Table 2). Many studies have shown that the roles of a specific gene in cancer development and progression differ based on the cancer type. For example, increased Notch levels were correlated with tumor grade and metastasis in CRC [15], whereas in glioblastoma, Notch signaling has tumor-suppressive effects [16]. Even in the same tumor cells, a gene may modulate cancer progression and suppression in an environment- or time-dependent manner [17, 18]. Although He et al. showed that NPC1L1-knockout mice had fewer tumors in colitis-associated tumorigenesis [12], the association between NPC1L1 expression and pathological features in

cancers has been poorly characterized. This may be because the expression of NPC1L1, a membrane protein, is low in organs other than the liver and small intestine, making it difficult to study. To determine why the roles of NPC1L1 in pathological features differ depending on the cancer type, it is necessary to study the proliferation, migration, and invasion of various cancer cells.

Abnormal lipid metabolism is pathologically associated with vascular disorders, hyperlipidemia, lipid storage-associated diseases, and obesity. Recent studies have shown that changes in lipid metabolism play an important role in the development and progression of cancer [19–21]. Lipids are used for energy supply, as structural materials for the cell membrane and signaling molecules, and for the post-transcriptional modification of cancer cells to promote cell proliferation and alter cell characteristics [19]. Studies have confirmed changes in the contents and compositions of fatty acids, oxylipins, and triacylglycerols in the serum, tumor tissues, and adipose tissue in patients with CRC [20], and shown that the molecules involved in altered lipid metabolism are potential biomarkers of CRC development, progression, and prognosis [21]. Among them, NPC1L1 is known to be an important molecule for cholesterol absorption and is associated with prognosis in patients with HCC [14]. Among patients with CRC in the TCGA cohort, the high NPC1L1 expression group showed poorer OS than the low NPC1L1 expression group (Fig. 3A). Consistent with the results of the first analysis, results of the analysis of the second cohort (GSE17536) also showed that high expression of NPC1L1 was associated with poor survival in patients with CRC (Fig. 3B). In the univariate and multivariate analyses of prognostic factors, NPC1L1 expression, along with age and disease stage, was significantly linked to OS in patients with CRC (Table 3). To confirm whether NPC1L1 represents an independent prognostic marker of CRC, the prognostic significance of NPC1L1, along with other CRC prognostic markers, such as VEGF-A, MACC1, and TGF-β1, was determined by multivariate regression analysis (Table 4). According to previous studies, the OS of CRC patients showing high expression of VEGF-A or MACC1 was significantly worse than that of CRC patients with low expression of VEGF-A or MACC1 [22, 23]. Based on a meta-analysis, Chen et al. showed that TGF-β may be used as a prognostic factor for patients with CRC [24]. In this study, we showed that NPC1L1 expression, along with VEGF-A expression, was significantly associated with the prognosis of CRC. Thus, our results suggest that NPC1L1, along with other known prognostic markers, may be an independent prognostic marker of CRC.

Drugs used to treat a disease can have several side effects, including cancer development. Statins, which are used to lower lipid levels, block the 3-hydroxy-3-methylglutaryl CoA reductase needed for cholesterol synthesis. According to previous studies, statins inhibit cell growth and induce apoptosis in CRC, thereby lowering the risk of gastrointestinal cancers, including CRC [25]. Ezetimibe, on the other hand, increased cancer incidence in the SEAS study (n = 1872), despite lowering the levels of lipids, similar to the effects of statins [26]. Consequently, the use of statin-ezetimibe combination therapy has been restricted in many guidelines. However, large-scale studies, such as SHARP (n = 9270) and IMPROVE-IT (n = 18144), showed that ezetimibe was not associated with cancer incidence [27, 28]. Thus, ezetimibe is currently used to treat uncontrolled dyslipidemia. These results suggest that it is necessary (for evidence-based medicine) to investigate the molecular mechanism underlying the action of NPC1L1 as the target of ezetimibe and to examine the association between NPC1L1 and the carcinogenesis and prognosis of cancers.

A limitation of this study is that we did not provide experimental evidence for the development and progression of CRC resulting from NPC1L1 overexpression; further, this study did not investigate the effects of ezetimibe, an inhibitor of NPC1L1, on the growth, invasion, and migration of CRC cells. Although our study does not provide any experimental evidence *per se*, it provides a framework for future studies to experimentally investigate the role of NPC1L1 at the molecular and cellular levels.

# Conclusion

NPC1L1 is upregulated in CRC and is associated with the stage and prognosis of CRC. Our study suggests that NPC1L1 may serve as an independent prognostic marker for CRC.

# List Of Abbreviations

AJCC, American Joint Committee on Cancer; CI, confidence interval; CRC, Colorectal cancer; GEO, Gene Expression Omnibus; HCC, hepatocellular carcinoma; MACC1, metastasis-associated in colon cancer 1; NPC1L1, Niemann-Pick C1-Like 1; OS, Overall survival; SD, standard deviation; TCGA, The Cancer Genome Atlas; TGFB1, transforming growth factor-beta 1; VEGF-A, vascular endothelial growth factor A.

# Declarations

### Ethics approval and consent to participate

This manuscript does not include studies with human participants or animals; the authors have used the public datasets available in the TCGA database.

### Consent for publication

The authors provide formal consent to publish this work.

### Availability of data and material

The datasets investigated and analyzed during this study are publicly available.

### Competing interests

The authors declare that they have no competing interests.

### Funding

This study was supported by the Research Institute for Convergence of Biomedical Science and Technology (30-2020-000), Pusan National University Yangsan Hospital.

### Authors' contributions

Conceptualization, R.J.K. and Y.H.C.; Formal analysis, R.J.K. S.M.S. and E.J.P.; Funding acquisition, Y.H.C.; Investigation, R.J.K., S.M.S., E.J.P. and Y.H.C.; Supervision, Y.H.C.; Validation, R.J.K., C.K., C.H. Y.J.R. and J.C.; Writing – original draft, R.J.K. G.L.K. and Y.L; Writing – review & editing, S.Y.L., Y.J.K., J.G.L., Y.H.Y., Y.J.T. and S.H.L. All authors read and approved the final version of the manuscript.

### Acknowledgements

The authors would like to acknowledge the Cancer Genome Atlas (TCGA) database and the tools provided by the TCGA database for data analysis.

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# Figures

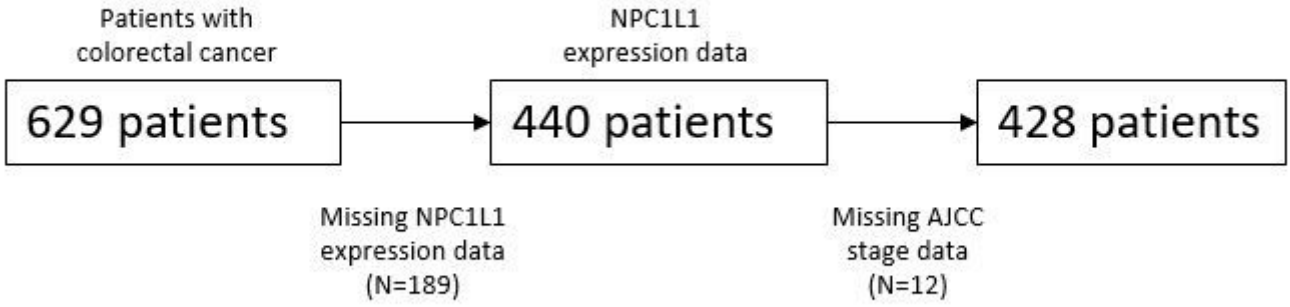
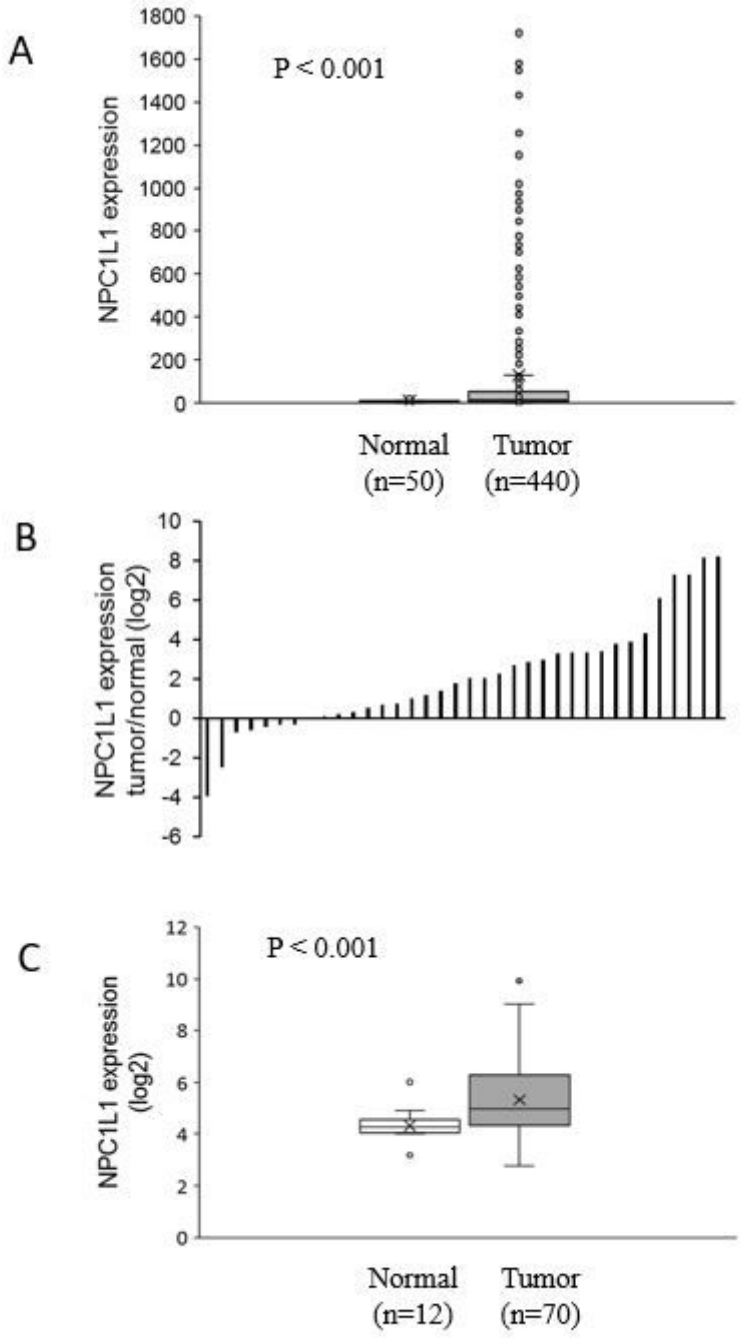



**Figure 1**

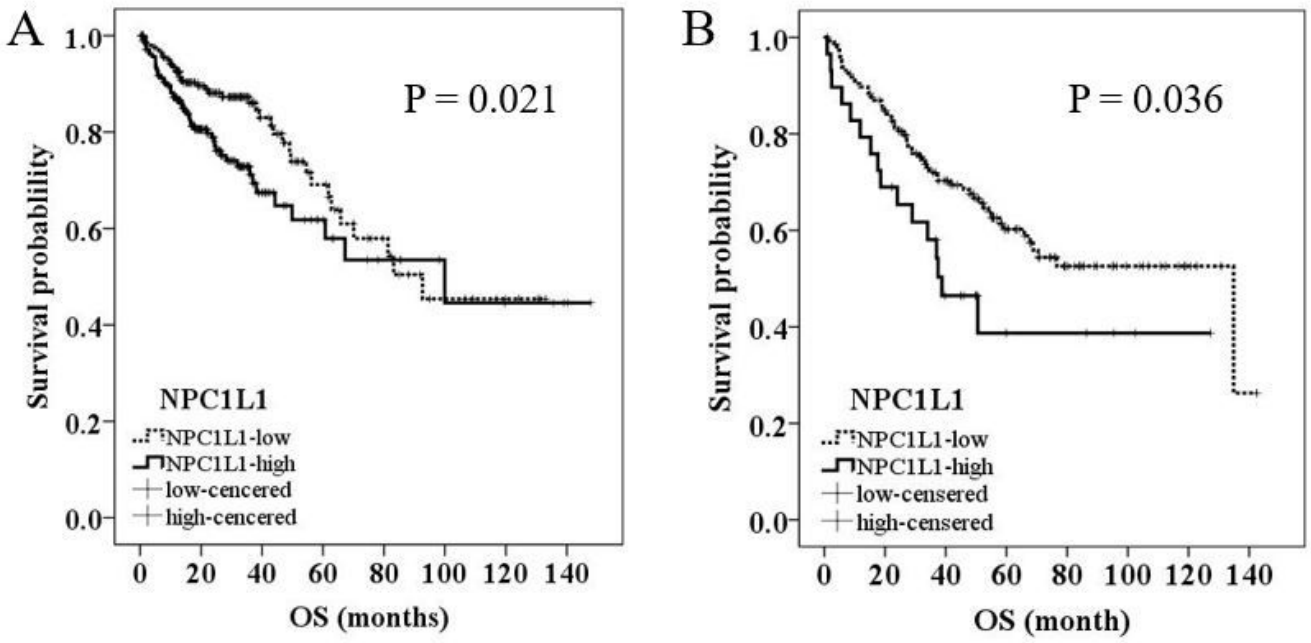
Flow chart used for selecting patients with colorectal cancer (CRC) for this study.




## Figure 2

NPC1L1 expression in CRC. (A) RNA expression values of NPC1L1 in CRC tissues compared to that in non-tumor tissues, as obtained from The Cancer Genome Atlas (TCGA) database, are presented in a box and whisker plot format. The numbers of non-tumor and tumor tissues analyzed were 50 and 440, respectively. Significance was measured using t-test ($p < 0.001$). (B) Relative expression levels of NPC1L1 in tumor tissues (CRCs) compared to that in paired non-tumor tissues are presented as a bar graph. The number of tumor and paired non-tumor tissues analyzed were 35 and 35, respectively. Significance was

measured using t-test ($p < 0.001$). (C) NPC1L1 expression in CRC tissues compared to that in normal tissues (GSE17536).




## Figure 3

Kaplan–Meier survival analysis based on the expression level of NPC1L1 (low, high). (A) Kaplan–Meier curve based on TCGA dataset. (B) Kaplan–Meier curve based on GEO dataset.