Identification of genomic alterations of perineural invasion in patients with stage II colorectal cancer

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Abstract

Background The molecular mechanism of perineural invasion (PNI) in stage Ⅱ colorectal cancer (CRC) remains not to be defined clearly. This study aims to identify the genomic aberrations related to PNI in stage Ⅱ CRC.

Methods Using array-based comparative genomic hybridization (array-CGH), primary tumor tissues and paracancerous normal tissues of stage Ⅱ CRC from 5 patients with PNI and 5 patients without PNI were analyzed. We also identified genomic aberrations by using Genomic Workbench and MD-SeeGH. Furthermore, Gene ontology (GO) and Pathway analysis for these array-CGH data was performed to determine the most likely biological effects of these genes.

Results The most frequent gains in stage Ⅱ CRC were at 7q11.21-q11.22, 8p11.21, 8p12-p11.23, 8q11.1-q11.22, 13q12.13-q12.2, and 20q11.21-q11.23 and the most frequent losses were at 17p13.1-p12, 8p23.2, and 118q11.2-q23. Four high-level amplifications at 8p11.23-p11.22, 18q21.1, 19q11-q12, and 20q11.21-q13.32 and homozygous deletions at 20p12.1 were discovered in Stage II CRC. Gains at 7q11.21-q22.1, 16p11.2, 17q23.3-q25.3, 19p13.3-p12, and 20p13-p11.1, and losses at 11q11-q12.1, 11p15.5-p15.1, 18p11.21, and 18q21.1-q23 were more commonly found in patients with PNI by frequency plot comparison together with detailed genomic analysis. It is also observed that gains at 8q11.1-q24.3, 9q13-q34.3, and 13q12.3-q13.1, and losses at 3q26.1, 8p23.3-p12, 17p13.3-p11.2, and 21q22.12 occurred more frequently in patients without PNI. GO and Pathway analysis revealed that the genes in two groups were enriched in specific pathways.

Conclusions These involved genomic changes in the PNI of stage Ⅱ CRC will contribute to reveal the mechanisms underlying PNI and provide candidate biomarkers.

Background

Colorectal cancer (CRC) has been ranked third in terms of cancer incidence and second in terms of cancer mortality, according to the International Agency for Research on Cancer (IARC) [1]. Management of CRC patients is commonly defined by the TNM stage at diagnosis, which is based on the depth of tumor wall invasion, lymph node involvement and distant metastasis [2]. However, the TNM stage alone does not accurately predict the prognosis and distinguish whether the patient should receive adjuvant chemotherapy, particularly in patients with stage II CRC. Among CRC, TNM stage II constitutes a very wide spectrum and the 5-year overall survival of surgically resected patients ranges between 75 and 80% [3–4]. Plenty of clinicopathological features have been associated with a high risk of recurrence and metastasis in stage II CRC, among which perineural invasion (PNI) has been associated with a poor outcome [5–7] and the postoperative survival rate of stage II CRC patients with PNI was supposed to be more similar to that of stage III [8].

Complex signaling between tumor cells, the nerves, and stromal cells is probably related to the pathogenesis of PNI [9–12].Several previous studies have identified that the overexpression of the ITGAV gene, the higher degree of PIWIL2 expression, the downregulated E-cadherin expression, CDX2 loss, and the loss of certain tight junction proteins, are associated with a higher progression and spread of CRC [13–15]. However, the molecular mechanism of PNI and the internal relation between PNI and tumor metastasis is still largely in its infancy, and related research has not been conducted in patients with stage Ⅱ CRC. Our interest is to detect frequent DNA copy number changes and identify genomic alternations in stage Ⅱ CRC patients with PNI. Array-based comparative genomic hybridization (array-CGH) has been used to the rapid genomic-wide screen for genetic aberrations such as gains and losses in solid tumors and proven to be a valuable and a convenient method. In the present study, the genomic alterations of both stage Ⅱ CRC with PNI and without PNI were investigated by array-CGH.

Methods

Tumor tissues

Fresh tumor tissues and corresponding paracancerous normal tissues from ten stage Ⅱ CRC patients in the department of Colorectal Surgery, Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, between 2014 to 2015, were included in this study. They were divided into two groups: a PNI group of five cases with PNI and a no perineural invasion (NPNI) group of five cases without PNI. Representative tumor regions and paracancerous normal tissues were excised and immediately stored at − 70℃ until use. Patients consisted of seven males and three females with an average age of 58.8 years (range, 46–71 years). None of the patients had received neoadjuvant therapy and all of them underwent radical operation (R0 resection).

The study protocol was approved by the Institutional Review Board for Human Use at Cancer Hospital, Chinese Academy of Medical Sciences, and informed consent for sampling and molecular analysis was obtained from all the patients. The clinicopathological characteristics of the patients in this study are summarized in Table 1.

Table 1
Clinicopathological characteristics of 10 patients

No.	PNI status	Sex	Age	pT1	pN2	cM3	Differentiation
1	PNI	F	71	3	0	0	Middle
2	PNI	M	60	3	0	0	Middle
3	PNI	M	73	3	0	0	Middle
4	PNI	M	56	4	0	0	High-middle
5	PNI	M	65	4	0	0	Middle
6	NPNI	F	60	4	0	0	Middle-low
7	NPNI	M	60	3	0	0	High-middle
8	NPNI	F	46	3	0	0	High
9	NPNI	M	51	3	0	0	Middle
10	NPNI	M	46	3	0	0	Middle

Note: M: male. F: female.

Array-based CGH analysis

According to the manufacturer's instructions (Qiagen, Hilden, Germany), the genomic DNA was isolated using the Qiagen DNeasy Blood & Tissue Kit from tumor tissues and the corresponding paracancerous normal tissues.

For each case, DNA from normal tissues was used as a reference for tumor DNA and all the DNA was digested with Alu I and RSA I restriction enzymes (PROMEGA, Warrington, UK). Array-based CGH analysis was carried out using standard Agilent protocols (Agilent Technologies, Santa Clara, CA). Briefly, 500–1000 ng of tumor DNA was labelled by cyanine-5 dUTP and the same amount of normal tissue-matched reference DNA was labelled by cyanine-3 dUTP (Agilent Technologies, Santa Clara, CA). The mixture and hybridization were performed in an Agilent 44K human genome CGH microarray (Agilent) for 40 h after clean-up. Then, the washing, scanning, and data extraction were performed as described earlier.

Microarray data analysis

A specially designed microarray reader system with software Agilent Genomic Workbench (Agilent Technologies, Santa Clara, CA) and MD-SeeGH (www.flintbox.ca), was used for analyzing the microarray

data. Agilent Genomic Workbench was used to calculate the $log2^{ratio}$ for every probe and to identify genomic aberrations. A mean $Log2^{ratio} > 0.75$ of all probes in a chromosome region was considered as a high-level DNA amplification, a mean $Log2^{ratio} > 0.25$ and ≤ 0.75 as a genomic gain, a mean $Log2^{ratio} < -0.25$ and ≥ -0.75 as a hemizygous loss, and a mean $Log2^{ratio} < -0.75$ as a homozygous deletion.

Gene Ontology and Pathway Analysis

The “clusterProfiler” package was recruited to perform the functional annotation of all significantly differentially expressed genes (DEGs), and Gene Ontology (GO) enrichment analysis including cellular component, molecular function, and biological processes was performed. In organisms, different genes coordinate with each other to exercise their biological functions. Pathway-based analysis was performed to further understand the biological functions of genes. The most important biochemical metabolic pathway and signal transduction pathway involved in genes was determined by significant enrichment of Pathways. The Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway was the main database for Pathway significance enrichment analysis.

Statistical analysis

Statistical analysis was performed with SPSS software, version19.0 for Windows (SPSS Inc., Chicago, IL, USA). Quantitative variables are given as the mean plus standard deviation and were compared by using the Student t-test, and qualitative variables were compared using the χ^2-test. A *p*-value lower than 0.05 was considered statistically significant.

Results

DNA copy number alterations in Stage II CRC

DNA copy number changes were detected in 9/10 (90%) of the Stage II CRC samples. Among them, less than forty genetic alterations were confirmed in five Stage II CRC cases (50%) and forty to eighty-four DNA copy number changes were revealed in four cases (40%, Fig. 1A). In addition, there was one case among the patients with PNI that had no DNA copy number changes. However, the number of DNA copy changes was not different between the two groups (Fig. 1B).

Thirteen gains and eighteen losses were frequently detected (frequency > 20%) in the analyzed samples of Stage II CRC. The most common gains were detected at 7q11.21-q11.22 (30%), 8p11.21 (30%), 8p12-p11.23 (30%), 8q11.1-q11.22 (30%), 13q12.13-q12.2 (30%), and 20q11.21-q11.23 (30%), and the most frequent losses were found at 17p13.1-p12(60%), 8p23.2 (40%), and 118q11.2-q23 (40%, Table 2 and Fig. 2). Four high-level amplifications were discovered at 8p11.23-p11.22, 18q21.1, 19q11-q12, and 20q11.21-q13.32 and homozygous deletions were seen at 20p12.1 in Stage II CRC (Table 3).

Table 2
Genomic gains and losses in Stage II CRC

Changes	No.	Cytoband	Start	End	case	Ave frequency
Gain	1	7q11.21 - q11.22	64139711	67496168	3	30%
	2	8p11.21	41574867	42914135	3	30%
	3	8p12 - p11.23	35608029	38105438	3	30%
	4	8q11.1 - q11.22	47512525	52559725	3	30%
	5	13q12.13 - q12.2	26889395	28813797	3	30%
	6	20q11.21 - q11.23	29991221	35929628	3	30%
	7	4q28.1 - q28.2	128792806	129099786	2	20%
	8	6p21.1	43703961	43867174	2	20%
	9	7p22.3 - p22.1	203985	7058843	2	20%
	10	7q21.3 - q22.1	97939894	100959652	2	20%
	11	9q33.3 - q34.2	127148150	136363110	2	20%
	12	11q23.3 - q24.3	115366301	129126621	2	20%
	13	13q22.1	73557740	73825937	2	20%
Lose	1	17p13.1 - p12	10293677	15343586	6	60%
	2	8p23.2	3333230	6043259	4	40%
	3	18q11.2 - q23	23338481	77992312	4	40%
	4	17q21.33 - q22	49658639	54428200	3	30%
	5	18p11.32 - p11.21	118760	14966054	3	30%
	6	1p36.21	14143854	15467335	2	20%
	7	2q11.2 - q12.1	99106249	104023375	2	20%
	8	4p15.1	31954751	34720163	2	20%
	9	4q21.1 - q24	77220752	106814071	2	20%
	10	8p21.1 - p12	27614256	30690240	2	20%
	11	10p15.3	2171195	2957100	2	20%
	12	10q23.2 - q23.31	89263612	90035024	2	20%

Note: when two or more adjacent cytobands have copy number changes at a frequency above 20%, the average frequency of these cytobands was calculated and listed.

Changes	No.	Cytoband	Start	End	case	Ave frequency
	13	15q11.2 - q13.1	23872298	29274461	2	20%
	14	16q12.2	53883354	54679642	2	20%
	15	18q21.2 - q21.31	48895772	53994773	2	20%
	16	20p12.3 - p12.1	6760377	16165894	2	20%
	17	22q11.22	22348912	23327667	2	20%
	18	22q12.3	32428769	35554956	2	20%

Note: when two or more adjacent cytobands have copy number changes at a frequency above 20%, the average frequency of these cytobands was calculated and listed.

Table 3
High-level amplifications and homozygous deletions in Stage II CRC

Changes	Cytoband	Start	End	No.of cases	Genes
AMP	4q31.3	153298167	154400562	1	FBXW7, MIR3140, DKFZP434I0714, TMEM154, TIGD4, ARFIP1, FHDC1, TRIM2, ANXA2P1, MND1, KIAA0922
	7q21.3 - q22.1	97626121	101939281	1	LMTK2, BHLHA15, TECPR1, BRI3, BAIAP2L1, NPTX2, TMEM130, TRRAP, MIR3609, ……, C7orf52, MOGAT3, PLOD3, ZNHIT1, CLDN15, FIS1, RABL5, EMID2, MYL10, CUX1, SH2B2, MIR4285
	8p11.21	40683161	41153818	1	ZMAT4, SFRP1
	8p11.23 - p11.22	38184189	39222426	2	WHSC1L1, LETM2, FGFR1, C8orf86, RNF5P1, TACC1, PLEKHA2, HTRA4, TM2D2, ADAM9, ADAM32, ADAM5P
	8p12	33028963	33444629	1	FUT10, MAK16, C8orf41, RNF122
	8q22.1 - q22.3	94310754	104490068	1	LOC642924, FAM92A1, RBM12B, C8orf39, TMEM67, PDP1, CDH17, GEM, RAD54B, ……, C8orf56, BAALC, MIR3151, LOC100499183, FZD6, CTHRC1, SLC25A32, DCAF13
	8q24.11 - q24.21	118813668	129152148	1	EXT1, SAMD12, TNFRSF11B, COLEC10, MAL2, ……, MYC, PVT1, MIR1204, MIR1205, MIR1206, MIR1207
	17q12 - q21.2	31830271	38943726	1	ACCN1, AA06, CCL2, CCL7, CCL11, CCL8, CCL13, CCL1, ……, KRT222, KRT24, KRT25, KRT26, KRT27
	17q24.1 - q24.2	63685275	66607651	1	CCDC46, APOH, PRKCA, MIR634, CACNG5, CACNG4,……, WIPI1, MIR635, PRKAR1A, FAM20A
	18q21.1	45953441	47143335	2	CTIF, SMAD7, LIPG
	18q21.2	50169570	50521636	1	DCC
	19q11 - q12	28272497	32171905	2	LOC148189, LOC148145, UQCRFS1, VSTM2B, POP4, PLEKHF1, C19orf12, CCNE1, C19orf2, ZNF536, DKFZp566F0947, TSHZ3, THEG5

Note: Amp: amplifications. HD: homozygous deletions.

Changes	Cytoband	Start	End	No.of cases	Genes
	20q11.21 - q13.32	29920027	57007584	2	DEFB118, DEFB119, DEFB121, DEFB122, DEFB123, DEFB124, REM1, ……, MTRNR2L3, RBM38, CTCFL, PCK1, ZBP1, PMEPA1, C20orf85, PPP4R1L, RAB22A, VAPB
	20p11.22 - p11.1	21419611	25732554	1	NKX2-2, PAX1, LOC284788, NCRNA00261, FOXA2, SSTR4, THBD, ……, PYGB, ABHD12, GINS1, NINL, NANP, ZNF337
	20p12.1 - p11.1	17078577	26194459	1	PCSK2, BFSP1, DSTN, RRBP1, BANF2, SNX5,……, FAM182B, LOC100134868, FAM182A, C20orf191, MIR663
	20q13.33	60543478	62752759	1	TAF4, LSM14B, PSMA7, SS18L1, GTPBP5, HRH3, ……, SOX18, TCEA2, RGS19, OPRL1, C20orf201, NPBWR2
HD	1q44	248738898	248785562	1	OR2T10
	4q32.2	162609927	163366981	1	FSTL5
	15q22.31 - q23	66944212	67891471	1	SMAD6, SMAD3, AAGAB, IQCH, C15orf61, MAP2K5
	17q21.33 - q22	49778999	50898782	1	CA10
	18q21.1	44094807	45739981	1	LOXHD1, ST8SIA5, PIAS2, KATNAL2, TCEB3CL, TCEB3C, TCEB3B, HDHD2, IER3IP1, SMAD2, ZBTB7C
	20p12.1	14734411	14774972	2	MACROD2
	22q11.23	24347959	24390254	1	LOC391322, GSTT1, GSTTP2
	22q13.33	51146403	51178264	1	SHANK3, ACR

Note: Amp: amplifications. HD: homozygous deletions.

Genomic changes associated with PNI in stage Ⅱ CRC

The genetic alterations linked with PNI status were analyzed by using the frequency plot comparison and significance analysis of microarrays (SAM) methods. Gains at 7q11.21-q22.1, 16p11.2, 17q23.3-q25.3, 19p13.3-p12, and 20p13-p11.1, and losses at 11q11-q12.1, 11p15.5-p15.1,18p11.21, and 18q21.1-q23 were found more commonly in the PNI group by using frequency plot comparison together with detailed genomic analysis. It is also observed that gains at 8q11.1-q24.3, 9q13-q34.3, and 13q12.3-q13.1, and losses at 3q26.1, 8p23.3-p12, 17p13.3-p11.2, and 21q22.12 ocurred more frequently in the NPNI group (Fig. 3).

GO and Pathways Enrichment

In order to determine the most likely biological effects of these genes, we performed GO analysis for these CGH data. GO analysis revealed that genes changed in stage CRC belonged to the classes of genes that participated in the following biological processes: organic substance biosynthesis, regulation of metabolic processes, molecular functions, regulation of macromolecule biosynthesis, binding biosynthetic process, regulation of macromolecule metabolic processes and metabolic processes (Fig. 4). We analyzed the genes of each of the two groups and found that the genes related to PNI mainly participated in DNA binding, olfactory receptor activity, sensory perception of smell, and biological processes. Meanwhile, the genes related to NPNI mainly belonged to homophilic cell adhesion via plasma membrane adhesion molecules, flavonoid glucuronidation, flavonoid biosynthetic processes and cellular glucuronidation (Fig. 5).

The related genes were annotated and enriched by Pathway Analysis, and it was found that the genes changed in stage CRC were mainly involved in the following pathways: signal transduction, gene expression, metabolism, immune system, metabolism of proteins, signaling by GPCR, generic transcription pathway, metabolic pathways, GPCR downstream signaling, and other basic metabolic processes. The KEGG pathway analysis revealed that these genes were mainly represented in metabolic pathways (Fig. 4). We also analyzed the genes of each of the two groups (see Fig. 5 for details).

Discussion

PNI was first described in a primary head and neck tumor in 1862 by Neumann and referred to as tumor invasion of nervous structures and spread along nerve sheaths [16]. With the development in the microanatomy of the peripheral cutaneous nerve, the definition of PNI has continued to change [17–18]. There are many different definitions of PNI used and there is still no agreement on a clear definition of PNI-positive. However, the broadest definition of PNI widely used in the literature is that tumor cells should surround > 33% of the nerve circumference without invading through the nerve sheath, as well as tumor cells within any of the 3 layers of the nerve sheath [19]. The incidence of PNI is reported to be 14–32% in CRC, which is much lower than pancreatic cancer (98%), cholangiocarcinomas (75%-85%), prostate (75%), and gastric cancer (60%) [20]. However, numerous reports have confirmed and quantified the strong negative prognostic impact for recurrence and survival in CRC when PNI is noted [21].

Due to the controversy regarding the issue of adjuvant therapy in stage II CRC patients, the prognostic significance of PNI in stage CRC appears to be particularly important in clinical practice [22]. Pathological features such as PNI, perforation, serosal extension, low tumor differentiation, low number of examined lymph nodes, venous or lymphatic invasion have been associated with a poor prognosis, thus, these patients may derive a potentially greater benefit from adjuvant chemotherapy [23]. Although it has been reported that stage CRC patients with PNI who received chemotherapy had a significantly

improved survival rate compared to those who did not [24], the target genes and molecular mechanisms underlying the association between PNI and stage Ⅱ CRC still remain unclear.

Using CGH, many studies investigated the genetic alterations in CRC and identified some chromosome regions and genes correlated with the carcinogenesis and tumor progression. It is known that the genomic changes of the adenoma–carcinoma sequence includes the activation of K-Ras and the inactivation of at least three tumor suppression genes, namely, loss of APC (chromosome region 5q21), loss of p53 (chromosome region 17p13), and loss of heterozygosity for the long arm of chromosome 18 (18q LOH) [25]. Interestingly, losses at 8p, 17p, 18p, and 18q and gains at 8q and 20q were reported to be observed in patients with CRC. Multiple high-level amplifications at 20q were also seen centering at 32.3, 37.8, 45.4, 54.7, 59.4, and 65 Mb [26]. Our study has revealed genetic gains at 7q11.21-q11.22, 8p11.21, 8p12-p11.23, 8q11.1-q11.22, 13q12.13-q12.2, and 20q11.21-q11.23, and losses at 17p13.1-p12, 8p23.2, and 118q11.2-q23 in Stage II CRC, some of which were consistent with the results of previous studies and some were special for Stage II.

In order to identify the genomic aberrations associated with PNI in Stage II CRC tissues, we used frequency plot comparison and SAM methods and found that gains at 7q11.21-q22.1, 16p11.2, 17q23.3-q25.3, 19p13.3-p12, and 20p13-p11.1, and losses at 11q11-q12.1, 11p15.5-p15.1, 18p11.21, and 18q21.1-q23 were more common in Stage II CRC patients with PNI. Meanwhile, gains at 8q11.1-q24.3, 9q13-q34.3, and 13q12.3-q13.1, and losses at 3q26.1, 8p23.3-p12, 17p13.3-p11.2, and 21q22.12 were more frequent in Stage II CRC patients without PNI. In fact, some studies have found a correlation between genomic alterations and PNI in CRC. Jin Cheon Kim et al. [27] confirmed that the gelsolin (GSN) gene at 9q33.2 was associated with PNI in CRC through the finding that the invasion potential was > 2-fold greater in GSN-overexpressing LoVo cells than in control cells. It was also reported that patients with a low GSN expression had a significantly higher 5-year recurrence-free survival (RFS) rate than those with GSN overexpression (73.6% vs. 64.7%, $p = 0.038$), which suggested its potential value as a predictor of recurrence or as a therapeutic target in CRC patients. It is found that CRC with PNI patients showed an overexpression of the ITGAV gene at 2q31-q32 compared to CRC without PNI patients ($p = 0.028$) and the expression of the corresponding ITGAV protein was also validated in that study ($p = 0.001$) [28]. Sun-Ju Oh et al. [29]revealed that there was a significant correlation between the high degree of PIWIL2 gene expression at chromosome 8 and PNI in CRC ($p = 0.027$) and PIWIL2 may contribute to a poor prognosis in CRC. The loss of the expression of paracellular tight junctions, claudin-1, -4, and − 7 were demonstrated to be related with PNI, tumor invasion depth, stage of the disease, tumor grade, lymphovascular invasion, and lymph node status in an investigative study [30]. Moreover, GO analysis confirmed that the genes related to PNI mainly participated in DNA binding and olfactory receptor activity. The Reactome pathway analysis revealed that these genes were mainly represented in the pathway of signal transduction, gene expression, and metabolism, suggesting that PNI may affect the prognosis of CRC through these processes and related target genes may belong to that pathway.

Conclusions

In conclusion, our data provide detailed genomic aberrations regarding PNI in Stage II CRC. Further studies are necessary to clarify the candidate target genes and to explore their implications in Stage II CRC.

Abbreviations

CRC: colorectal cancer; IARC: International Agency for Research on Cancer; PNI: perineural invasion; array-CGH: array-based comparative genomic hybridization; NPNI: no perineural invasion; DEGs: differentially expressed genes; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; SAM: significance analysis of microarrays; GSN: gelsolin; RFS: recurrence-free survival.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board for Human Use at Cancer Hospital, Chinese Academy of Medical Sciences, and informed consent for sampling and molecular analysis was obtained from all the patients.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

SH carried out CGH experiments, participated in the data analyses, and draft the manuscript. CH carried out GO analysis. HJJ organized clinicopathological information. XX organized clinicopathological

information. BMDL carried out part of the CGH experiments. LS performed some statistical analysis. ZCD performed some statistical analysis. LQ gave experimental suggestions. WXS provided the statistical analysis suggestion. ZZX participated in the design of the study and gave experimental design suggestions. ZHT conceived of the study and participated in its design. All authors have read and approved the final manuscript.

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Figures

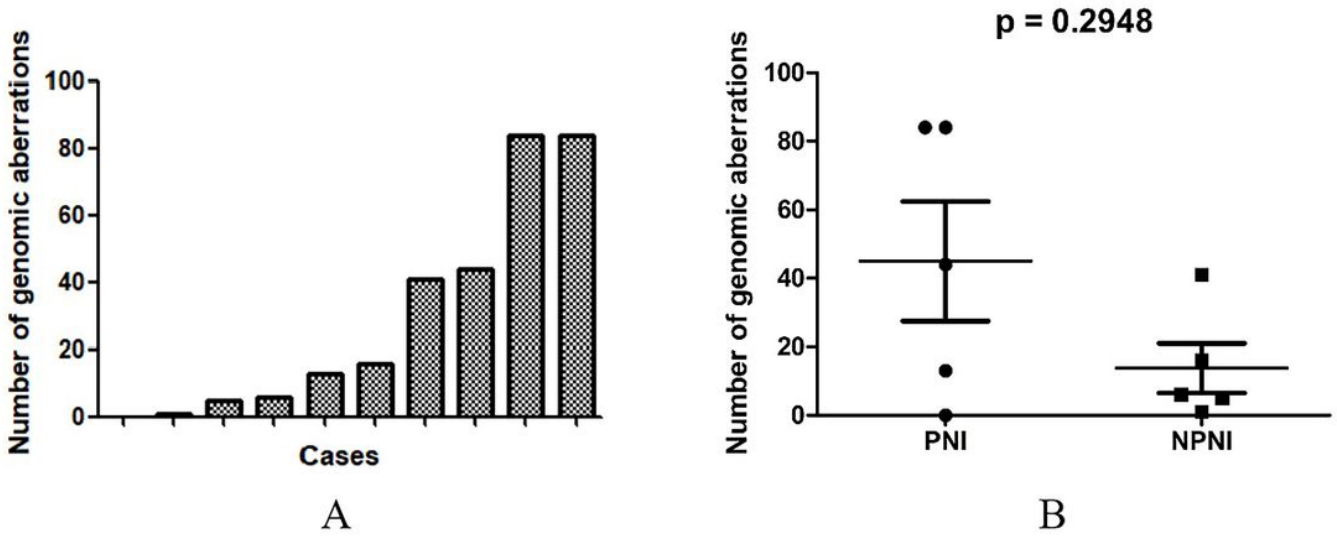

Figure 1

Number of genomic aberrations in Stage II CRC. A. Number of genomic aberrations per case; B. Comparison of numbers of genomic aberrations between the PNI and NPNI groups.

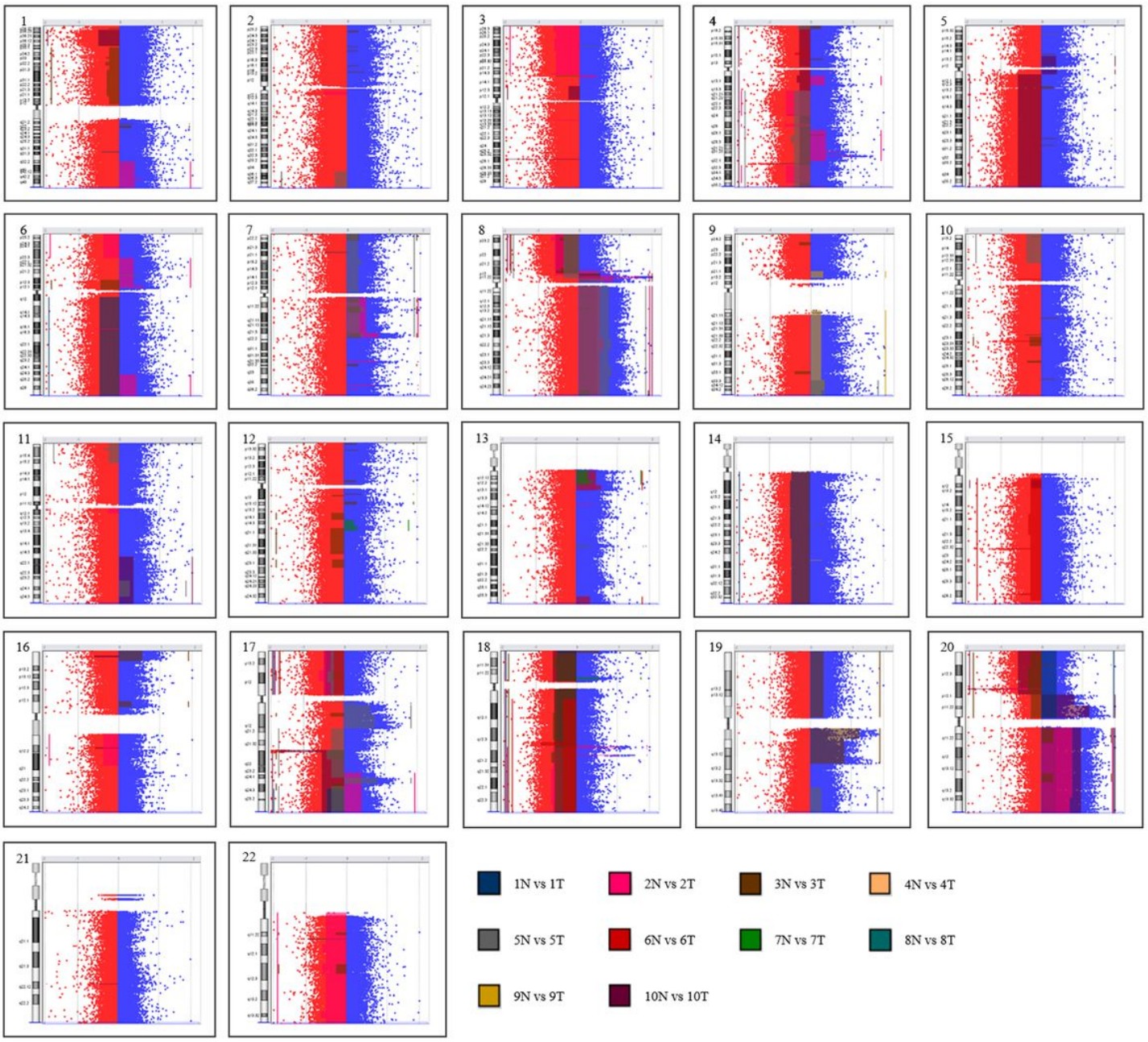

Figure 2

Signal values and differential fragment distribution maps of all samples. Red dots denote losses, blue dots denote gains, and blocks of different colors represent different sample pairs.

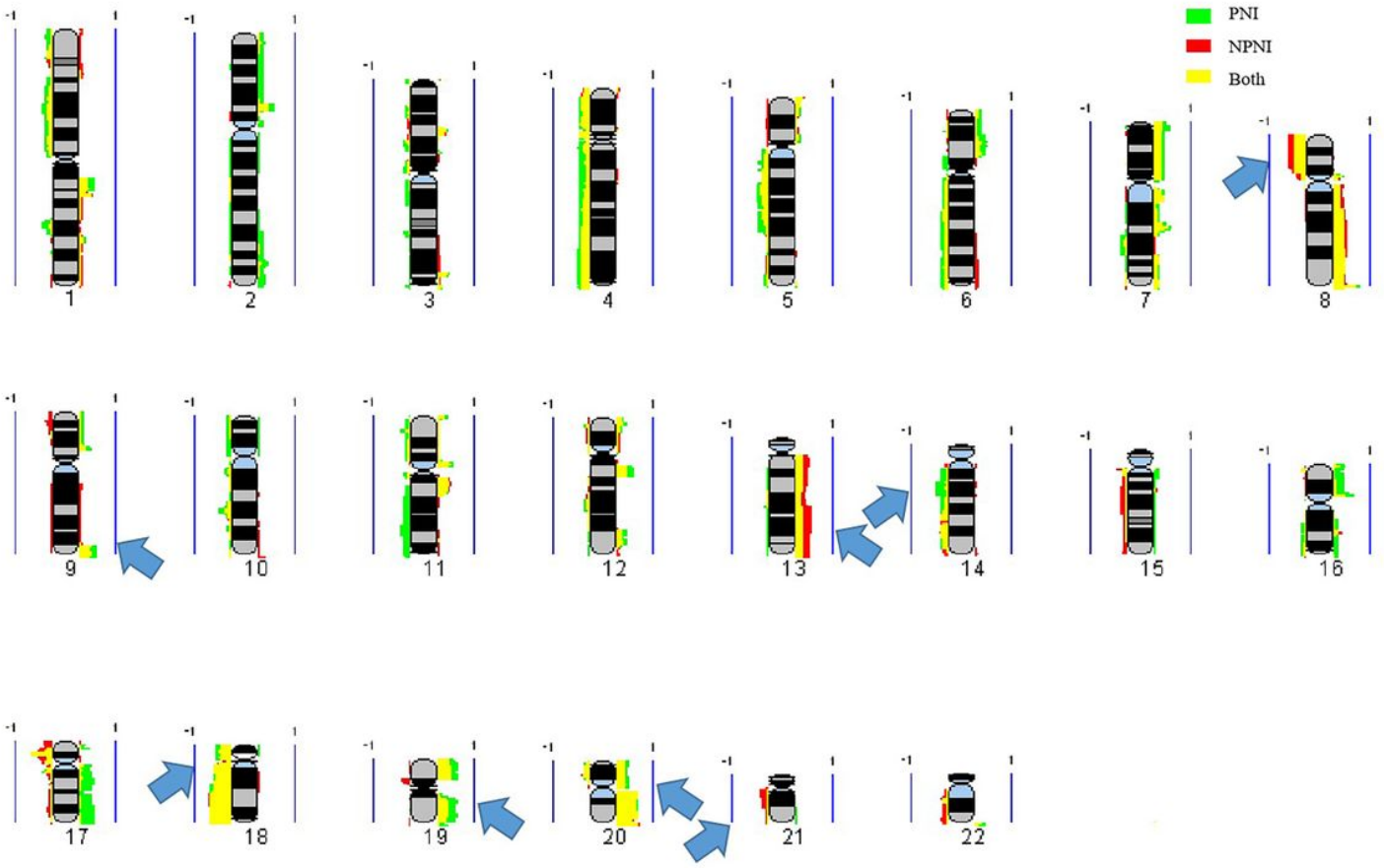

Figure 3

Frequency plot comparison in the two groups. Green, frequency plot of genomic changes in the PNI group; red, frequency plot of genomic changes in the NPNI group; yellow, genomic changes shared by the two groups. The presentation is per array probe: gains are represented by the lines on the right, and losses by the lines on the left. The vertical line represents 100% of the samples. The arrows highlight the chromosomal areas with different frequency in the two groups.

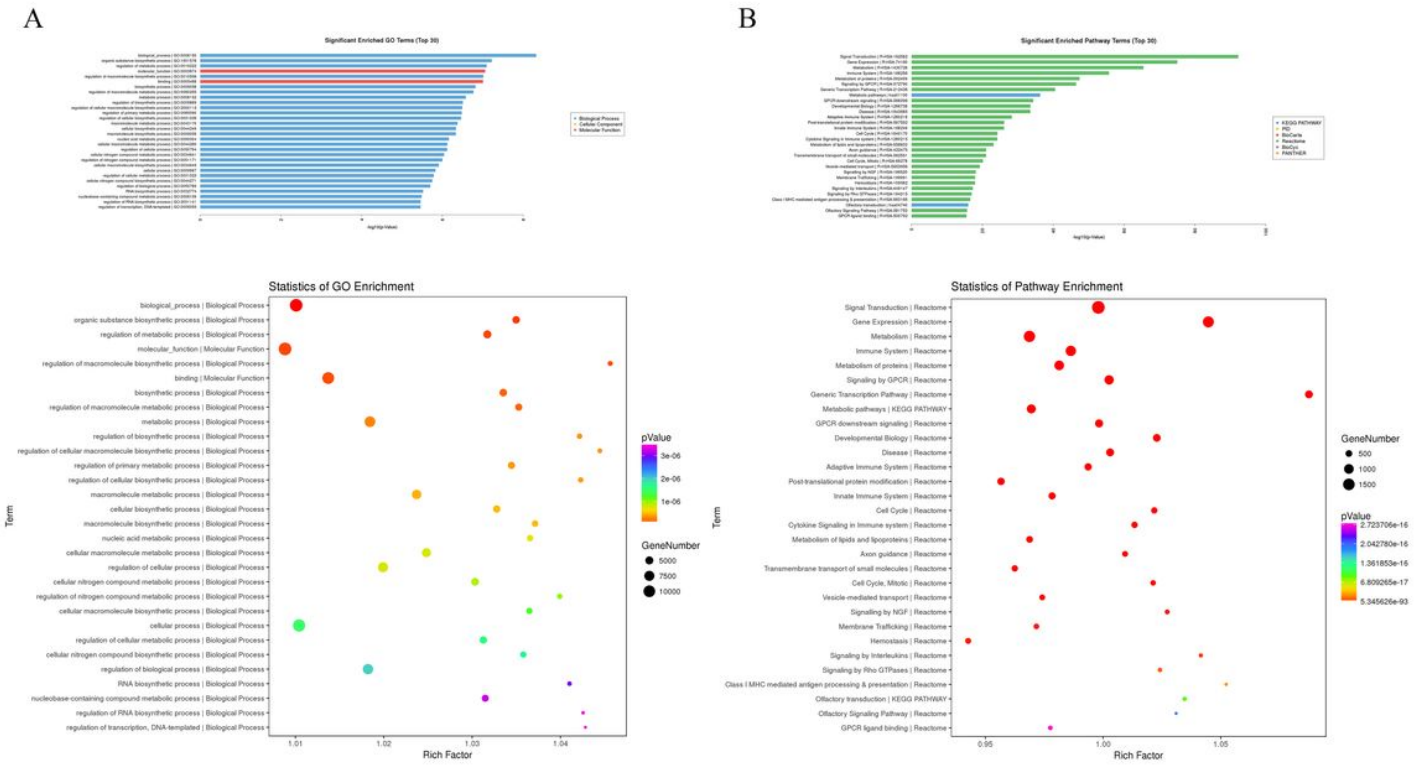

Figure 4

Data of the GO and Pathways Enriched in Stage II CRC. A. GO Enrichment; B. Pathways Enrichment. The bar chart selects the top 30 terms with the lowest p-value in the enrichment results to draw the enrichment path diagram. The enrichment factor map corresponds to the bar chart, and the data are derived from the enrichment results.

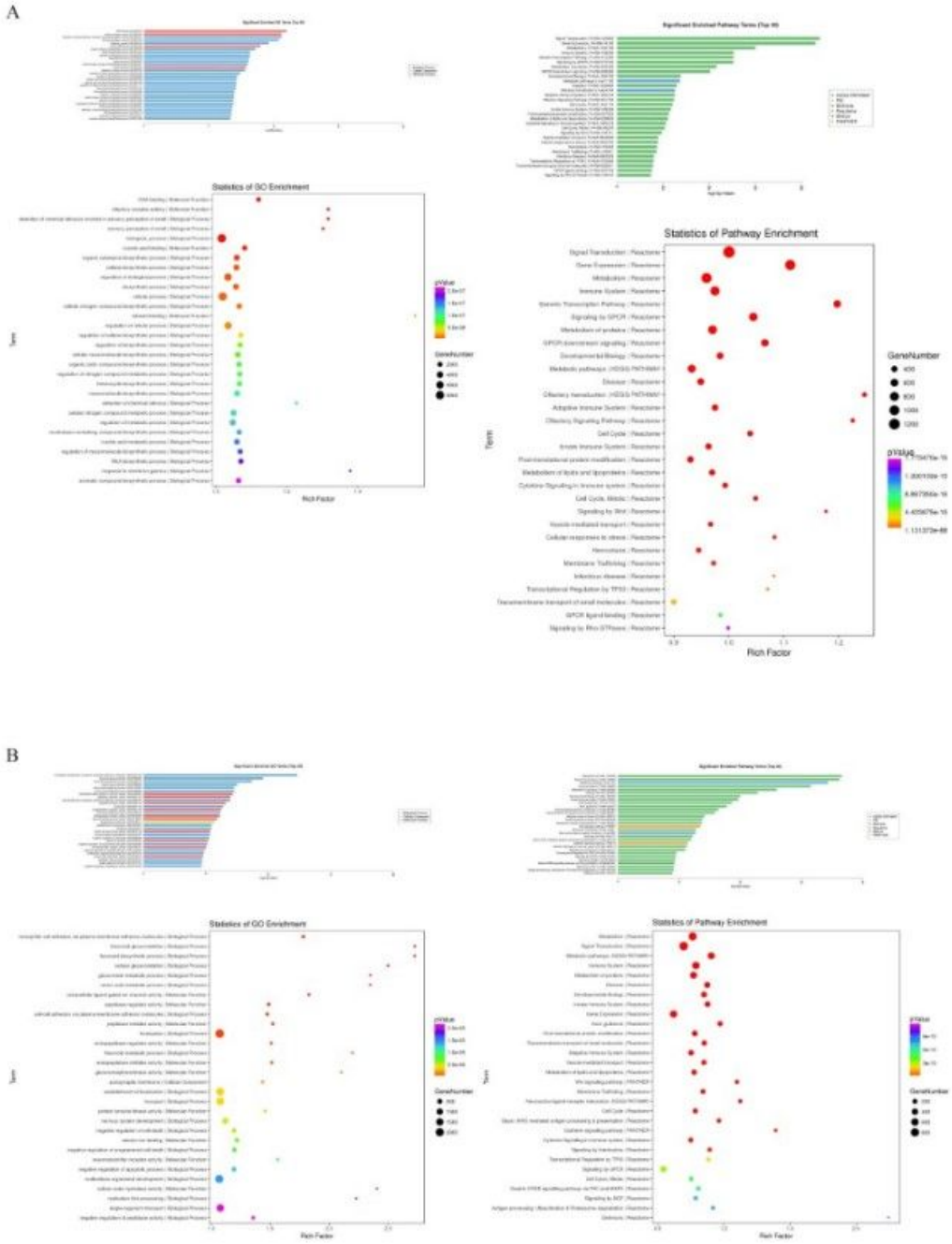

Figure 5

GO and Pathways Enriched in the two groups. A. GO and Pathways Enrichment in the PNI group; B. GO and Pathways Enrichment in the NPNI group; The bar chart selects the top 30 terms with the lowest p-value in the enrichment result to draw the enrichment path diagram. The enrichment factor map corresponds to the bar chart, and the data are derived from the enrichment results.