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Original Article

Application of molecular diagnosis in individualized treatment of colorectal carcinoma

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Abstract: Colorectal carcinoma is the third most common malignant tumor in digestive tract, the prognosis of which is associated with several tumor biomarkers. Chemotherapy is one of the primary treatment methods for colorectal carcinoma. This study tailored individualized treatment for colorectal carcinoma based on different molecular targets, and evaluated the safety and efficacy of the treatment. A total of 180 colorectal cancer patients were recruited for individualized treatment, who were given individualized treatment based on molecular diagnostic results of different therapeutic targets, whereas the control group (n=180) was administered standard FOLFOX protocol. Post-treatment serum level of tumor markers including CEA, CA19-9 and sTK1 were examined. Clinical symptoms were compared before and after treatment to evaluate treatment efficacy. Adverse effects and 3-year survival condition were recorded. When compared with controls, individualized group had significantly lower serum levels of CEA, CA19-9 and sTK1 after treatment ($P<0.05$) and higher treatment efficacy ($P<0.05$). Adverse effects, 1-year and 2-year survival rate in both groups were similar ($P>0.05$). The individualized group had lower cumulative mortality and higher 3-year survival rate compared with controls ($P<0.05$). The individualized group, however, had lower bone marrow suppression rate ($P<0.05$). Molecular diagnosis can be used to determine the specific individualized treatment, and to assess the treatment efficacy. Individualized treatment can improve the prognosis of colorectal cancer, and offers an effective and safe method for clinical treatment of the malignant disease.

Keywords: Molecular diagnosis, colorectal carcinoma, individualized treatment

Introduction

Colorectal carcinoma is the third most common malignant tumor in digestive tract, only next to gastric carcinoma and esophageal cancer [1, 2]. With the transition of life styles in recent years, the incidence and mortality rate of colorectal carcinoma is gradually increasing, and the age of onset is becoming much younger [3-5]. Chemotherapy remains one of the major approaches for the treatment of middle-late stage colorectal carcinoma [6-8]. Recently, individualized treatment based on the characteristics of different tumor cells has become a research hotspot in tumor treatment. Individualized treatment is tailored based on the disease progression and gene/protein expression profiles of each patient in order to reach an optimal treatment efficacy and minimal adverse effects. Such approach can therefore greatly improve the efficacy, elongate the survival time, and even lower the mortality rate. Individualized treatment can also notably reduce the financial burden on patients.

The relationship between individualized treatment and efficacy/prognosis is critical. Individualized treatment group have major advantages in judging biological behavior, early diagnosis, drug selection, treatment efficacy and prognosis evaluation [10]. The molecular diagnosis approach, including treatment efficacy and adverse effects has not been well studied and thus requires replenishment of evidences. Several biomarkers have been known to be closely associated with the occurrence, progression and prognosis of colorectal carcinoma. For instance, tumor biomarkers CEA, CA19-9 and sTK1 are barely expressed in healthy cells, but are highly expressed in malignant tumor cells [9]. This study compared the expression level of these tumor markers before and after treatment, in order to establish a batch of tumor molecule tags related to the efficacy of individu-

Table 1. Selection of different drug targets and samples

Drug		Target and index	Samples
Chemotherapy drug	Oxaliplatin	ERCC1 expression level	Paraffin slice/block, formalin-fixed tissue
		ERCC1 (118C>T) gene polymorphism	Anti-coagulant blood samples
		XRCC1 (R399Q) gene polymorphism	Anti-coagulant blood samples
	5-Fu/capecitabine	TS expression level	Paraffin slice/block, formalin-fixed tissue
	Irinotecan	TOPO I expression level	Paraffin slice/block, formalin-fixed tissue
		UGT1A1*28 gene polymorphism	Anti-coagulant blood samples
Targeted drugs	Bevacizumab	ICAM expression level	Anti-coagulant blood samples
		VEGFR2 phosphorylation level	Paraffin slice/block, formalin-fixed tissue
	Cetuximab	EGFR expression level	Paraffin slice/block, formalin-fixed tissue
		KRAS gene mutation	Paraffin slice/block, formalin-fixed tissue
		PI3KA gene mutation	Paraffin slice/block, formalin-fixed tissue
		BRAF gene mutation	Paraffin slice/block, formalin-fixed tissue
		PTEN expression level	Paraffin slice/block, formalin-fixed tissue

alized treatment. This study will shed lights on the clinical implication of molecular diagnosis in the individualized treatment of colorectal carcinoma.

Material and methods

Research objects

This study included a total of 360 colorectal carcinoma patients in Mingzhou Hospital of Ningbo from March 2012 to March 2014. All patients had experienced no prior treatment, and had normal heart, lung and kidney functions. Patients were randomly divided into individualized treatment and control group (n=180 each). The patients in both groups had no significant difference in sex ratio, age, Dukes stage and differentiation grade. The study protocol was approved by the Research Ethics Committee of Mingzhou Hospital of Ningbo. All patients were required to sign the informed consent.

Research methods

In addition to routine blood indexes, all patients were examined for serum CDA, CA-19, and sTK1 level, chest X-ray, abdominal ultrasound, electrocardiography and enteroscopy after being admitted. Samples including paraffin-based tissue block, formalin-fixed tissues, and anticoagulant treated blood were collected from individualized group and subjected to molecular test for therapeutic targets of commonly used chemotherapy or targeted drugs (**Table 1**). Individualized treatment with one targeted drug or two sensitive chemotherapy drugs was determined for each patient based on the molecular examination results according to the USA National Comprehensive Cancer Network (NCCN), the UK National Institute for Health and Clinical Excellence (NICE), and the national guidelines for colorectal cancer by the China Ministry of Health (**Table 1**). The control group was given standard FOLFOX therapy, which included 100 mg/m^2 oxaliplatin (iv drip) on day 1,200 mg/m^2 calcium folinate (iv drip) from day 1 to 5, and 500 mg/m^2 fluorouracil (iv drip) on from 1 to 5. The chemotherapy was repeated for 6 times in every 4 weeks. Follow-up was performed each month to observe major symptoms, body sign, imaging (X-ray, CT, MRI, ultrasound), tumor markers (CEA, CA19-9, sTK1) and adverse effects (neurotoxicity, gastrointestinal response, bone marrow suppression, liver/kidney damage).

Treatment efficacy

Short-term efficacy was assessed based on treatment efficacy evaluation criteria (WHO), Patients was considered complete remission (CR), partial remission (PR), stable disease (SD) or progressed disease (PD). Among those, CR+PR were regarded as effective treatment, whose percentage was defined as response rate (RR). According to the grading criteria of common toxicity/adverse effects of anti-tumor drugs, the adverse effects were divided into 0, I, II, III and IV grade, with grade 0 for no chemotoxicity and grade IV for the most potent adverse effects. The toxicity and adverse effects of chemotherapy drugs mainly included digestive response, hematological system response and peripheral neurotoxicity. Digestive response includes nausea, vomiting and diar-

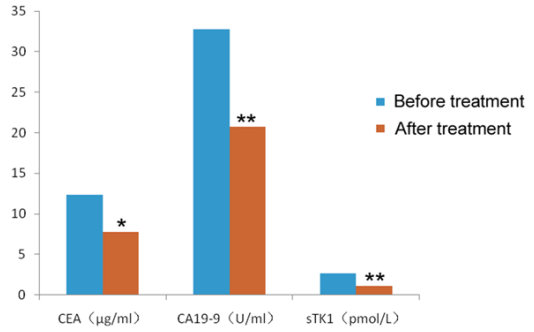

Figure 1. Comparison of pre- and post-treatment serum index in individualized treatment group. *, P<0.05 and **, P<0.01 compared with pre-treatment value.

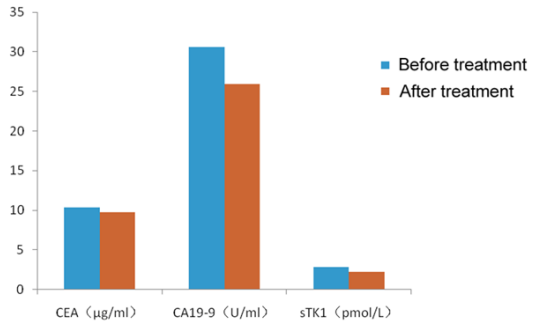

Figure 2. Comparison of pre- and post-treatment serum index in control group.

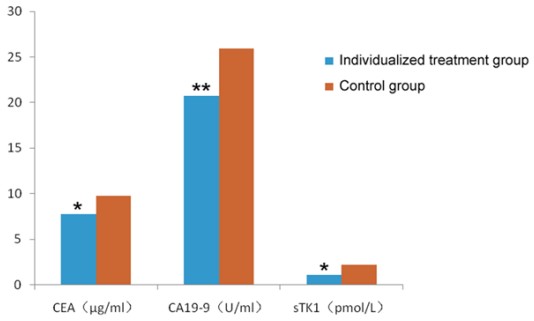

Figure 3. Comparison of post-treatment serum index in individualized treatment and control groups. *, P<0.05 and **, P<0.01 compared with control group.

rhea. Hematological toxicity included bone marrow inhibition, as shown by down-regulation of leukocytes. Progression-free-survival (PFS) refers to the time elapsed from first chemotherapy to disease progression or death. Follow-ups were performed by out-patient clinics or telephone interviews. The last follow-up was performed on March 2015.

Statistical analysis

All data were analyzed by SPSS19.0 software package. Measurement data were presented as mean ± standard deviation and were compared by student t-test. Enumeration data were compared by chi-square test. Survival rate was analyzed by Kalan-Meier approach and log-rank test. *P* values smaller than 0.05 were considered statistically different.

Results

Serum index

A total of 180 cases of colorectal cancer patients had decreased level of serum CEA, CA19-9 and sTK1 after individualized treatment (P<0.01 or 0.05, **Figure 1**). The cohort controls who received FOLFOX plan had unchaned post-treatment level of those indexes (P>0.05, **Figure 2**). Moreover, the serum CEA, CA19-9 and sTK1 level in individualized group was significantly lower compared with control group (P<0.01 or 0.05, **Figure 3**).

Short-term treatment efficacy

All patients had evaluable short-term treatment efficacy and no one had CR. In individualized group, there were 60 patients with PR, 72 with SD and 48 with PD, resulting in a RR of 33.3%. In control group, there were 42 PR patients, 76 SD and 62 PD cases, leading to a RR of 23.3%. The RR in individualized group was significantly higher compared with the control group (P< 0.05).

Toxicity and adverse effects

As shown in **Table 2**, the incidence of digestive response including nausea and vomiting in individualized and control group was 26.1% and 31.6%, respectively (P>0.05). The incidence of peripheral neurotoxicity in individualized and control group was 18.9% and 25.0%, respectively, with no statistical significance (P>0.05). The rate of bone marrow suppression in individualized group (17.2%) was significantly lower than that in controls (27.2%, P<0.05).

Long-term treatment efficacy

Follow-up studies showed that the median PFS in individualized and control group was 2.6 years (95% CI, 1.5~3 years) and 2.1 years (95%

Table 2. Comparison of toxicity and adverse effects between treatment groups

Individualized treatment						Control					
	I	II	III	IV	Rate	I	II	III	IV	Rate	*P* value
Vomiting/nausea	25	13	9	0	26.1%	31	16	10	0	31.6%	0.25
Bone marrow suppression	27	4	0	0	17.2%	42	6	1	0	27.2%	0.02
Peripheral neurotoxicity	30	3	1	0	18.9%	38	6	1	0	25.0%	0.16

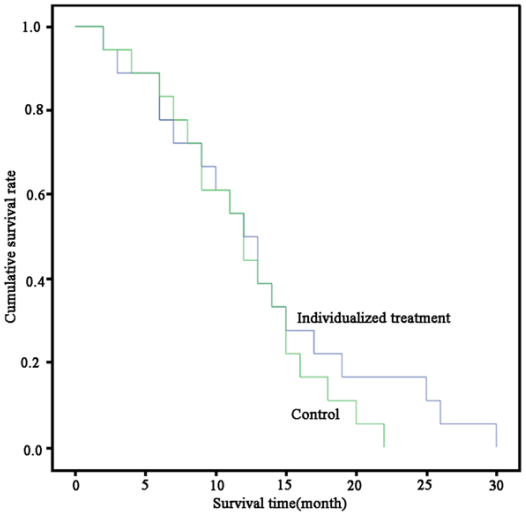

Figure 4. Survival curve of individualized treatment and control patients.

CI, 1.6~2.8 years), respectively. The survival curve was shown in **Figure 4**. No significant difference in 1-year and 2-year survival rate was found between individualized and control groups (P>0.05). The individualized group, however, had lower cumulative mortality and higher 3-year survival rate compared with controls (P<0.05, **Table 3**).

Discussion

Currently, detection of serum tumor markers such as CEA, CA19-9 and sTK1 has been a common molecular diagnosis method for colorectal carcinoma. Several studies have shown that the level of these markers, especially CEA is closely associated with the disease condition of colorectal cancer [11-13]. The combined assay of serum tumor markers can be used for the diagnosis and prognosis of colorectal cancer, making them important clinical prognostic index for the tumor. In this study, serum levels of CEA, CA-19-9 and sTK1 levels were all decreased in individualized group, suggesting the treatment efficacy. Control group had only marginal but statistically insignificant decrease in serum level of these markers after the treatment. Furthermore, the post-treatment serum CEA, CA-19-9 and sTK1 levels in individualized group were significantly lower compared with control group, suggesting a higher treatment efficacy of individualized treatment for colorectal cancer.

Chemotherapy is one of the major treatment approaches for middle-late stage colorectal carcinoma. It has multiple adverse effects including nausea, vomiting, abdominal distension, decreased leukocytes, anemia, thrombocytopenia, dizzy, liver dysfunction, kidney failure and rash [14-16]. Therefore, the establishment of treatment plan with less adverse effects can largely relieve pains and improve life quality. This study found relatively lower bone marrow suppression rate in individualized treatment patients, suggesting less bone marrow toxicity and benefiting for treatment. No significant difference, however, has been found regarding digestive tract response and peripheral neurotoxicity, suggesting that neither chemotherapy plan induced digestive tract adverse effects such as nausea, vomiting and neurotoxicity. In clinics, anti-vomiting drugs and neural nutrition agents can be used to prevent such toxicity and alleviate adverse effects. This study also found that the individualized group had lower cumulative mortality and higher 3-year survival rate compared with controls (P<0.05). These results have collectively suggested that individualized treatment can decrease the mortality rate and extend the survival period of colorectal carcinoma patients, and thus is superior to the standard chemotherapy.

Molecular diagnosis has been used to help to select sensitive or targeted drugs in treating colorectal cancer. Individualized drugs had higher specificity, less adverse effects, and thus more treatment benefits. Previous studies have found that cetuximab, one epithelial growth factor receptor inhibitor, was effective for colorectal carcinoma patients carrying KRAS wild type genes, but not for those with mutant form of KRAS gene [17]. This study optimized the chemotherapy plan based on the examination

Table 3. Survival rate of all groups after treatment

Group	Cumulative mortality (5%)	1-year survival rate (%)	2-year survival rate (%)	3-year survival rate (%)
Individualized treatment	21.7 (39/180)$^{\Delta}$	86.7 (156/180)	75.0 (135/180)	67.2 (121/180)$^{\Delta}$
Control	36.1 (65/180)	77.2 (139/180)	65.6 (118/180)	52.2 (94/180)

Note: $^{\Delta}P<0.05$ compared to control group.

results of molecular targets, and thus achieved improved efficacy.

Although combined therapy including surgery, chemotherapy, radiotherapy, biotherapy and Chinese traditional medicine have been developed for the treatment of colorectal cancer [18, 19] and the survival rate of patients has been notably improved, the prognosis and survival period of patients are still far from being satisfactory. Therefore, the optimization of treatment plan based on unique features of each patient is essential to reach a better treatment outcome [20]. This study tailored individualized treatment strategy with optimal chemotherapy drugs and monitored treatment efficacy, adverse effects, and survival rates of patients based on molecular diagnosis, providing new insights into the application of molecular diagnosis in the individualized treatment of colorectal carcinoma.

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Disclosure of conflict of interest

None.

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References

[1] Mousa L, Salem ME, Mikhail S. Biomarkers of Angiogenesis in Colorectal Cancer. Biomark Cancer 2015; 7 Suppl 1: 13-9.

[2] Zhang B, Jia WH, Matsuda K, Kweon SS, Matsuo K, Xiang YB, Shin A, Jee SH, Kim DH, Cai Q, Long J, Shi J, Wen W, Yang G, Zhang Y, Li C, Li B, Guo Y, Ren Z,Ji BT, Pan ZZ, Takahashi A, Shin MH, Matsuda F, Gao YT, Oh JH, Kim S, Ahn YO; Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO), Chan AT, Chang-Claude J, Slattery ML; Colorectal Transdisciplinary (CORECT) Study, Gruber SB, Schumacher FR, Stenzel SL; Colon Cancer Family Registry (CCFR), Casey G, Kim HR, Jeong JY, Park JW, Li HL, Hosono S, Cho SH, Kubo M, Shu XO, Zeng YX, Zheng W. Large-scale genetic study in East Asians identifies six new loci associated with colorectal cancer risk. Nat Genet 2014; 46: 533-42.

[3] Nagtegaal ID, Hugen N. The Increasing Relevance of Tumour Histology in Determining Oncological Outcomes in Colorectal Cancer. Curr Colorectal Cancer Rep 2015; 11: 259-266.

[4] Koga Y, Yamazaki N, Matsumura Y. Fecal Biomarker for Colorectal Cancer Diagnosis. Rinsho Byori 2015; 63: 361-8.

[5] Lim D, Ha M, Song I. Trends in major cancer mortality in Korea, 1983-2012, with a joinpoint analysis. Cancer Epidemiol 2015; 39: 939-946.

[6] Tampellini M, Polverari RS, Ottone A, Alabiso I, Baratelli C, Bitossi R, Brizzi MP, Leone F, Forti L, Bertona E, Racca P, Mecca C, Alabiso O, Aglietta M, Berruti A, Scagliotti GV. Circannual variation of efficacy outcomes in patients with newly diagnosed metastatic colorectal cancer and treated with first-line chemotherapy. Chronobiol Int 2015; 32: 1359-66.

[7] Ando K, Oki E, Saeki H, Kasagi Y, Tsuda Y, Zaitsu Y, Nakashima Y, Imamura YU, Ohgaki K, Maehara Y. Number of Lymph Node Metastases May Indicate the Regimen for Adjuvant Chemotherapy in Patients with Stage III Colorectal Cancer. Anticancer Res 2015; 35: 6207-11.

[8] Feng L, Liu Y, Wu X, Liu Q, Xia D, Xu L. Safety evaluation of intraoperative peritoneal chemotherapy with Lobaplatin for advanced colorectal cancers. Zhonghua Wei Chang Wai Ke Za Zhi 2015; 18: 1006-10.

[9] Zhong W, Yu Z, Zhan J, Yu T, Lin Y, Xia ZS, Yuan YH, Chen QK. Association of serum levels of CEA, CA199, CA125, CYFRA21-1 and CA72-4 and disease characteristics in colorectal cancer. Pathol Oncol Res 2015; 21: 83-95.

[10] Cho WC. Molecular diagnostics for monitoring and predicting therapeutic effect in cancer. Expert Rev Mol Diagn 2011; 11: 9-12.

[11] Bagaria B, Sood S, Sharma R, Lalwani S. Comparative study of CEA and CA19-9 in esophageal, gastric and colon cancers individually and in combination (ROC curve analysis). Cancer Biol Med 2013; 10: 148-57.
[12] Pei H, Pei Q, Wu S, Zhu H. Sensitivity study on preoperative individual concomitant radiochemotherapy for rectal cancer. Zhonghua Wei Chang Wai Ke Za Zhi 2014; 17: 565-9.
[13] Suárez J, Marín G, Vera R, Oronoz B, Oteiza F, Mata E. Complications from the primary tumour are not related with survival in patients with synchronous stage IV colorectal cancer receiving chemotherapy without primary tumour resection. Int J Colorectal Dis 2015; 30: 1357-63.
[14] Irving GR, Iwuji CO, Morgan B, Berry DP, Steward WP, Thomas A, Brown K, Howells LM. Combining curcumin (C3-complex, Sabinsa) with standard care FOLFOX chemotherapy in patients with inoperable colorectal cancer (CUFOX): study protocol for a randomised control trial. Trials 2015; 16: 110.
[15] Cohen Z, Maimon Y, Yoeli-Lerner M, Yang P, Samuels N, Berger R. Selective anticancer effects and protection from chemotherapy by the botanical compound LCS101: Implications for cancer treatment. Int J Oncol 2015; 46: 308-16.
[16] Altaf R, Lund Brixen A, Kristensen B, Nielsen SE. Incidence of cold-induced peripheral neuropathy and dose modification of adjuvant oxaliplatin-based chemotherapy for patients with colorectal cancer. Oncology 2014; 87: 167-72.
[17] Bertotti A, Papp E, Jones S, Adleff V, Anagnostou V, Lupo B, Sausen M, Phallen J, Hruban CA, Tokheim C, Niknafs N, Nesselbush M, Lytle K, Sassi F, Cottino F, Migliardi G, Zanella ER, Ribero D, Russolillo N, Mellano A, Muratore A, Paraluppi G, Salizzoni M, Marsoni S, Kragh M, Lantto J, Cassingena A, Li QK, Karchin R, Scharpf R, Sartore-Bianchi A, Siena S, Diaz LA Jr, Trusolino L, Velculescu VE. The genomic landscape of response to EGFR blockade in colorectal cancer. Nature 2015; 526: 263-7.
[18] Harty GT, Jarrett J, Jofre-Bonet M. Consequences Of Biomarker Analysis On The Cost-Effectiveness Of Cetuximab In Combination With Irinotecan Based Chemotherapy For First-Line Treatment Of Metastatic Colorectal Cancer. Stratified Medicine At Work? Value Health 2015; 18: A456.
[19] Yamazaki Y. Metabolome Analysis of Human Serum: Implications for Early Detection of Colorectal Cancer. Rinsho Byori 2015; 63: 328-35.
[20] Lin BQ, Wang RL, Li QX, Chen W, Huang ZY. Investigation of treatment methods in obstructive colorectal cancer. J BUON 2015; 20: 756-61.