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-
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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 stTK1 level, chest X-ray, abdominal ultrasound, electrocardiography and enteroscopy after being admitted. Samples including paraffin-based tissue block, formalin-fixed tissues, and anti-coagulant treated blood were collected from individualized group and subjected to 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/m² oxaliplatin (iv drip) on day 1, 200 mg/m² calcium folinate (iv drip) from day 1 to 5, and 500 mg/m² 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, stTK1) and adverse effects (neurotoxicity, gastrointestinal response, bone marrow suppression, liver/kidney damage).

Table 1. Selection of different drug targets and samples

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target and index</th>
<th>Samples</th>
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<tbody>
<tr>
<td><strong>Chemotherapy drug</strong></td>
<td></td>
<td></td>
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<tr>
<td>Oxa</td>
<td>ERCC1 expression level Paraffin slice/block, formalin-fixed tissue</td>
<td></td>
</tr>
<tr>
<td>liplatin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ERCC1 (118C&gt;T) gene polymorphism Anti-coagulant blood samples</td>
<td></td>
</tr>
<tr>
<td></td>
<td>XRCC1 (R399Q) gene polymorphism Anti-coagulant blood samples</td>
<td></td>
</tr>
<tr>
<td>5-Fu/capecitabine</td>
<td>TS expression level Paraffin slice/block, formalin-fixed tissue</td>
<td></td>
</tr>
<tr>
<td>Irinotecan</td>
<td>TOP1 expression level Paraffin slice/block, formalin-fixed tissue</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UGT1A1*28 gene polymorphism Anti-coagulant blood samples</td>
<td></td>
</tr>
<tr>
<td><strong>Targeted drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>ICAM expression level Anti-coagulant blood samples</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VEGFR2 phosphorylation level Paraffin slice/block, formalin-fixed tissue</td>
<td></td>
</tr>
<tr>
<td>Cetuximab</td>
<td>EGFR expression level Paraffin slice/block, formalin-fixed tissue</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KRAS gene mutation Paraffin slice/block, formalin-fixed tissue</td>
<td></td>
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<tr>
<td></td>
<td>PI3KA gene mutation Paraffin slice/block, formalin-fixed tissue</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BRAF gene mutation Paraffin slice/block, formalin-fixed tissue</td>
<td></td>
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<tr>
<td></td>
<td>PTEN expression level Paraffin slice/block, formalin-fixed tissue</td>
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</table>
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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 unchanged 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%
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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 2. Comparison of toxicity and adverse effects between treatment groups

<table>
<thead>
<tr>
<th></th>
<th>Individualized treatment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Vomiting/nausea</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td>Bone marrow suppression</td>
<td>27</td>
<td>4</td>
</tr>
<tr>
<td>Peripheral neurotoxicity</td>
<td>30</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 4. Survival curve of individualized treatment and control patients.
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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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Table 3. Survival rate of all groups after treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Cumulative mortality (5%)</th>
<th>1-year survival rate (%)</th>
<th>2-year survival rate (%)</th>
<th>3-year survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individualized</td>
<td>21.7 (39/180)</td>
<td>86.7 (156/180)</td>
<td>75.0 (135/180)</td>
<td>67.2 (121/180)</td>
</tr>
<tr>
<td>Control</td>
<td>36.1 (65/180)</td>
<td>77.2 (139/180)</td>
<td>65.6 (118/180)</td>
<td>52.2 (94/180)</td>
</tr>
</tbody>
</table>

Note: *P<0.05 compared to control group.

References


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