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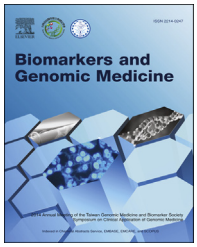

SHORT COMMUNICATION

Predictive biomarkers for the efficacy of concurrent chemoradiotherapy for patients with colorectal cancer

Hsin-Ju Wu [a], Hsueh-Chiao Liu [a,b], Yu-Tang Chang [c], Shiu-Ru Lin [d,*], Long-Sen Chang [a,**]

[a] *Institute of Biomedical Science, National Sun Yat-Sen University, Kaohsiung, Taiwan*
[b] *Division of Laboratory Medicine, Fooying University Hospital, Pingtung, Taiwan*
[c] *Division of Pediatric Surgery, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung, Taiwan*
[d] *Division of Medical Research, Fooying University Hospital, Pingtung, Taiwan*

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Abstract Colorectal cancer is a common gastrointestinal malignancy. Radiation combined with chemotherapy (also known as concurrent chemoradiotherapy or CCRT) is often used prior to surgery for treating severe cases of colorectal cancer. However, responses of individual tumors to CCRT differ. Therefore, in light of the variability in radiation sensitivity among different tumors, identifying the factors that can be applied to predict CCRT efficacy prior to treatment will aid in making decisions regarding an appropriate treatment strategy. In the present study, we used a gene chip to analyze the expression of candidate genes in the tumor cells of colorectal cancer patients prior to and after treatment with CCRT, in order to identify molecular markers that can predict the efficacy of CCRT. First, we selected a total of 15 CCRT candidate genes based on the results of previous studies, which used the microarray method to select CCRT response-related genes that were also related to tumor malignancy. We collected preoperative CCRT tumor tissues from 17 colorectal cancer patients for whom the efficacy of CCRT had already been determined and used gene chips to analyze the expression of CCRT-related genes in the tissues of these patients. We then compared the results for the expression of CCRT-related genes with those for the clinical efficacy of CCRT. Of the 15 candidate genes, five genes (*CK-20*, *ELAVL4*, *EV12B*, *TM4SF3*, and *ATPA2*) were upregulated in >29.4% and one gene (*MET*) was downregulated in 23.5% of the total patients after

* Corresponding author. Division of Medical Research, Fooyin University Hospital, Number 5, Jhongshan Road, Donggang Township, Pingtung 928, Taiwan.
** Corresponding author. Institute of Biomedical Sciences, National Sun Yat-Sen University, 70 Lienhai Road, Kaohsiung 804, Taiwan.
E-mail addresses: srlin@ms2.hinet.net (S.-R. Lin), lschang@mail.nsysu.edu.tw (L.-S. Chang).

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treatment with CCRT, indicating that these genes may be potential predictive markers for CCRT efficacy.

Introduction

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths. Most cancer treatments are primarily based on surgical resection, but surgery is not suitable for treating all types of cancers (for example, in cases where the tumor has spread) due to some limitations. As such, radiation therapy or chemotherapy is often used as an adjuvant therapy. Effectiveness of radiotherapy treatment is not limited by the lesion location. Preoperative radiation therapy usually yields better results by shrinking the tumor, thus increasing the chances of complete surgical resection of the tumor. Therefore, a combination of comprehensive surgery and radiotherapy can provide local control to improve treatment efficacy.[1–3] According to the 2010 United States National Comprehensive Cancer Network Version 1 guidelines, radiation prior to surgery combined with chemotherapy (CCRT) is the recommended treatment option for locally advanced CRCs (T3—4 or N1—2). Studies have reported that the role of chemical treatment drugs (such as 5-fluorouracil) in preoperative CCRT for patients with advanced or unresectable CRCs is to enhance the effectiveness of radiation therapy in killing cancer cells. In other words, radiation therapy provides the major therapeutic effects of CCRT, whereas chemotherapy supplements those therapeutic effects.[4–6]

Clinically, approximately 30—40% of patients are unable to obtain the benefit of a decreased tumor stage (downstaging) after receiving CCRT. In patients for whom CCRT show poor efficacy, preoperative CCRT is more harmful than helpful because it not only delays the timing of tumor resection, but also results in immunosuppression, causes patients to suffer unnecessarily, and is a waste of medical resources. As such, if we can predict the efficacy of CCRT, we can use medical resources and treat the disease more effectively. Such predictive ability would thus not only reduce the chances of recurrence, but also greatly enhance the likelihood of organ retention, particularly the affected organs.

The reason for individual differences in sensitivity to CCRT is that different tumors exhibit different expression profiles of related genes. Because cellular responses rely, in part, on changes in gene expression, the extent to which the radiation-responsive genes are induced or repressed influences how the cells deal with radiation exposure, where individual variability in radiation sensitivity is observed at the gene expression level.[7–9] Therefore, in the present study, we sought to determine the molecular markers that can predict the efficacy of CCRT, and then explored the impact of each genetic marker using a chip platform that was established by Wang et al.[10] This platform possesses the various advantages of biochips, has a lower cost, and exhibits better usability, higher reproducibility, and higher accuracy compared to traditional chips. Results obtained with this technology are consistent with the real-time quantitative polymerase chain reaction test results. Therefore, it is possible to predict the efficiency of clinical treatment by establishing a chip system; these prediction results may serve as important references and help clinical doctors in treatment planning prior to initiation of therapy.

Materials and methods

Collection of specimen

In our study, data were gathered for 17 patients for whom the clinical efficacy of CCRT was already known, excluding any tissue specimens from CRC patients who also had other cancers at the same time. For each patient, a biopsy sample of the tumor tissue was taken prior to and after treatment with CCRT prior to surgery, and kept on ice or in liquid nitrogen. All the patients received preoperative radiotherapy (45—50 Gy), and all were also treated with chemotherapy during the radiotherapy process. All the patients then subsequently underwent surgery. Data on the clinical characteristics of each patient, including patient age, gender, tumor size, location, histological type, clinical stage, metastasis situation, and other information, were collected.

Selection of genes and oligonucleotide design

Fifteen candidate genes were selected based on the microarray research of our previous studies and other recent studies[11,12] in which the microarray method was used to compare the expressions of candidate genes in radiosensitive and radiation-resistant cells, as well as to determine which genes are related to tumor malignancy. Using the Oligo Explorer software program (Gene Link, Inc., New York, NY, USA), we designed a detection chip to identify the nucleic acid sequences of candidate genes related to radiotherapy effects.

Preparation of gene chip

The synthetic oligonucleotides were dissolved at a concentration of 10 nM in double distilled water. We then used BioDOT AD1500 (BioDot Inc., Irvine, CA, USA) to automatically dot candidate genes, B-actin (positive control), and dimethyl sulfoxide (blank) on nylon membrane in triplicate. After quick drying and adhesive fixing, the prototype chip was obtained.