Screening of prognostic biomarkers for Stereotactic Body Radiation Therapy in liver cancer

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Abstract

So far there are still no effective immediate-early markers for assessing the efficacy of Stereotactic Body Radiation Therapy (SBRT). To find effective biomarkers for accurate assessment of the efficacy of SBRT in patients with liver cancer. Patients with liver cancer were included at Ruikang Hospital affiliated to Guangxi Medical University from January 2012 to December 2018. Follow-up was conducted, clinical information and blood samples (before SBRT, before discharge and 2 months after SBRT) were collected. mRNAs profiles were detected by high-throughput sequencing, followed by qPCR verification. The commonly-used serum biomarkers such as AFP, CEA and CA125 shown low prognostic value in distinguishing survival group and death group, indicated by low AUC (less than 0.7) and Yoden indexes (less than 0.5). Based on high-throughput sequencing of test group and qPCR detection of another verification group, we found 16 up-regulated and 12 down-regulated genes after SBRT. Among them, ADIPOR1 and EPB42 showed significantly different between effective and ineffective group after SBRT, ROC suggested that based on the optimal threshold of 0.5838, ADIPOR1 shown a sensitivity of 100% and a specificity of 83.33% to distinguish effective from ineffective group. Similarly, EPB42 had a sensitivity of 75% and a specificity of 100% at the optimal threshold of 1.3817. Thus, ADIPOR1 and EPB42 in whole blood are promising candidates to act as prognostic biomarkers for predication of SBRT outcomes in liver cancer patients.

Introduction

Liver cancer is expected to be the sixth most frequently diagnosed cancer and the fourth leading cause of cancer death around the world in 2018, with 841,000 new cases and 782,000 deaths per year[1]. It is also one of the most common malignant tumors in China, showing No. 4 of morbidity and No. 2 of mortality among malignant tumors in Chinese[2]. The main risk factors for liver cancer are chronic hepatitis B virus (HBV) or hepatitis C virus (HCV), heavy alcohol intake, aflatoxin-contaminated foods, obesity, smoking, etc. Among all cancers in China, liver cancer has the poorest survival and the age-standardized 5-year relative survival is only 10.1%[3]. Invasion, metastasis and recurrence are the primary factors that affect clinical treatment and prognosis[4]. Besides, the insidious onset of liver cancer is another reason, for the majority of liver cancer patients are diagnosed at a late stage when it is too far advanced to be cured[5]. Being highly malignant with rapid progression, the treatment for advanced liver cancer is difficult.

At present, there are various treatment methods for liver cancer, including surgical treatment, such as radical surgical resection and liver transplantation; non-surgical treatment, such as local ablation therapy, arterial chemoembolization, gene molecular targeted therapy, systemic chemotherapy, radiotherapy and et[6]. Surgical resection has proven to be the optimal treatment for long-term survival of liver cancer patients[7]. However, more than 70% of patients with liver cancer are unable to undergo liver resection due to the location, size, number of liver tumors, and impaired liver function. Therefore, the status of non-surgical therapy in the treatment of liver cancer is self-evident. For patients with liver cancer who are unable to undergo surgery, the National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology (NCCN guidelines) recommend radiation therapy as one of the treatments[8].

With the rapid development of computer, radiotherapy and imaging technology, precise radiotherapy has become possible. Beginning with the three-dimensional conformal radiation therapy, radiation therapy is increasingly being used for the treatment of liver cancer. At present, radiotherapy for liver cancer includes a series of advanced technologies, such as intensity-modulated radiation therapy, body stereotactic radiotherapy, and particle therapy. Current precision external exposure techniques ensure that the tumor is locally administered with high doses while protecting the remaining normal liver tissue from exposure to low doses, thereby limiting the risk of radiation-induced liver damage. In addition, external beam radiation therapy (EBRT) is applied to tumors in almost all locations of the liver. Stereotactic Body Radiosurgery (SBRT) is an advanced technique of EBRT that delivers large ablative doses of radiation. Increasing evidence supported the usefulness of SBRT for patients with unresectable, locally advanced, or recurrent liver cancer[9–11]. Additionally, NCCN also recommends that SBRT can also be used as an alternative to ablation/TACE treatments, options for treatment after ablation/ TACE failure, or treatment options for liver cancer patients with ablation/ TACE contraindications.

However, to date, the evaluation of radiotherapy efficacy relies mainly on imaging data and the calculated local control based on complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD), such long-term index as overall survival (OS), disease-free survival (DFS), progression-free survival (PFS), etc are also used. In addition, some serum molecules have been used for diagnostic marker, for example, AFP has long been used for diagnosis of liver cancer, but even in the advanced stage 15–30% of patients with a normal AFP levels[12]. So far there are still no effective immediate-early markers for assessing the efficacy of radiotherapy. Therefore, finding biomarkers with prognostic value for radiotherapy is still a focus that researchers are paying attention to. Genetics affects the occurrence and development of tumors, whether the change of mRNA profiles in venous blood after radiotherapy could be used as prognostic biomarkers, is our concern, this is the first report for searching prognostic biomarkers for liver patients treated by SBRT.

Material And Methods

Patient selection and medical record collection

The liver cancer patients involved in this study were divided into two parts. In the retrospective study, the clinical records of all liver cancer patients who underwent SBRT at Ruikang Hospital affiliated to Guangxi Medical University from January 2012 to December 2018 were retrospectively reviewed and follow-up was conducted in the following years. In the prospective study, we collected blood samples from liver cancer patients who received SBRT from December 2017 to December 2018. Three blood samples for each patient were collected, i.e., before SBRT, before discharge and 2 months after SBRT. In addition, the inclusion criteria are as follows: 1. Size less than 10cm, with Child A or B liver function; 2. First treatment without previous treatment history, 80Gy < BED < 100Gy (BED: Biologically Effective Dose); 3. No other comprehensive treatment such as chemotherapy that seriously affects the blood index. Patients were all informed of the study and signed a written informed consent form. All patients provided informed written consent and all research and related activities involving human subjects were approved by the Ethics Committee of the 1st and 2nd hospital affiliated to Jilin University and performed in accordance with guidelines and regulations and the Declaration of Helsinki. Trial registration: ResMan, number: ChiCTR1800015499. Registered 20 April 2018, www.medresman.org

Specimen collection and High-throughput sequencing

In this study, Paxgene Blood RNA Tubes (PreAnalytiX, Qiagen BD, Valencia, CA) (abbreviated as BRT) were used to collect blood samples for quick protection of the RNA from degradation. PAXgene Blood RNA Kit (PreAnalytiX, Qiagen BD, Valencia, CA) was used for subsequent experimental studies. The whole blood samples (5mL) were transported to Novogene (Novogene, Beijing) for RNA isolation, quality control, library preparation and sequencing.

Quantification of mRNAs by RT-qPCR analysis

Blood samples were collected before SBRT (first sample), before discharge (second sample) and 2 months after SBRT (third sample), for a total of 51 samples. Blood sample collection and RNA extraction were all carried as previously described. For the reverse transcription reaction, the RT reaction solution was prepared on ice according to the following components: 2 μl of 5×PrimeScript RT Master Mix (Perfect Real Time), 500 ng of total RNA, followed by RNase Free dH2O up to 10 μl. The reaction mixtures were incubated at 37 °C for 15 min, followed by 85 °C for 5 sec and saved at 4 °C. qRT-PCR was performed using the ABI StepOnePlus^{TM} Real-Time PCR System (Applied Biosystems, Foster City, CA).

All primer set were designed and synthesized by Wcgene Biotechnology Corporation (Shanghai, China). The sense and antisense primers of ADIPOR1 are: TCCTGCCAGTAACAGGGAAG and GGTTGGCGATTACCCGTTTG; the sense and antisense primers of EPB42 are: ACTTGTTGAA CCAGAATGGT CTC and TCCACTTCTC TACCTGCTTG TC; and so on. GAPDH (forward primers: CAATGACCCC TTCATTGACC and reverse primers: GACAAGCTTC CCGTTCTCAG) was used as the reference control. We used the TB Green^{TM} Premix Ex TaqTM II (Tli RNaseH Plus) (Takara, Code No. RR820A) as the QPCR test kit following the manufacturer's recommendations. The $2^{-\Delta\Delta Ct}$ method was performed to calculate the relative levels of mRNAs.

Statistical analysis

Nonparametric test and Student's t test were used to compare differences between two groups. Area under receiver-operating characteristic (ROC) curve (AUC) was used to determine the optimal cut-off value of blood mRNA levels and their diagnostic ability. *P*-values (in two-sided tests) ≤ 0.05 were considered statistically significant. Histograms were plotted by Graphpad Prism 7.0 (San Diego, California). Heatmap was drawn by the pheatmap package within R package version 3.5.1. Statistical analyses were performed using SPSS, version 24 (IBM Corp., Armonk, NY, USA). In addition, Medcalc software, version 11.5.0.0 (MedCalc Software, Mariakerke, Belgium) was used to evaluate the prognostic value of biomarkers. All authors had access to the study data and had reviewed and approved the final manuscript.

Results

Patient specimens

In the retrospective study, we collected a total of 589 liver cancer patients admitted to hospital for SBRT between January 2012 and December 2018. After excluding 55 patients who were lost for follow-up, a total of 534 patients were involved in the following research. In the prospective study, 51 samples were collected, the outcome after SBRT were followed up and recorded, the relationship between markers and prognosis, the ROC and assessment of prognostic value were analyzed.

Prognostic analysis of commonly-used serum tumor biomarkers

Cancer biomarkers, such as AFP, CEA, CA199, CA153, CA125, are commonly used for diagnosis. By detecting the levels of these biomarkers, clinician can make a preliminary assessment of the occurrence and progress of tumors. Jung J et al.[13] reported that liver cancer patients had a better prognosis when AFP levels returned to normal levels 3 months after SBRT, Uemotok et al.[14] found that the risk of recurrence was associated with the elevated AFP level. Whether those biomarkers are with prognostic value is our concern. In this study, we selected patients together with serum biomarker examination before SBRT, 3 months after SBRT, and 6 months after SBRT. The results are shown in **Table 1.** We found that AFP, CA125 and CA199 were differential expressed before and after SBRT.

Then, we wanted to figure out the relationship between these markers and the overall survival (OS) for 1-year, 2-year, and 3-year (**Table 2)**. From Table 2, significant differences were found between the survival group and the death group, i.e., change ratio of AFP in 6 months after SBRT, and CA125 in 3 months and 6 months after SBRT in 1-year, 2-year and 3-year OS. And no difference was found between the survival group and the death group in the change of CA199.

The diagnostic ability of these markers were then evaluated. The ROC curve was made and the sensitivity, specificity, AUC (Area Under the ROC Curve), Youden index and cut-off values were shown in **Table 3**. The Yoden index of AFP, CA125 and CA199 as prognostic markers for judging the efficacy of SBRT was less than 0.5. In addition, the AUC values of the change ratio of tumor biomarkers distinguishing survival group and death group were almost less than 0.70, except for CA125 in 6 months which were 0.781 and 0.715 in 1-year group and 2-year group, respectively, indicating that these commonly-used biomarkers do not provide a good assessment of the therapeutic efficacy of SBRT. Therefore, it is necessary to find more effective biomarkers for accurate assessment of the efficacy of SBRT in patients with liver cancer.

High-throughput sequencing for gene profiles

By the high-throughput sequencing, we obtained gene expression profiles before and after SBRT. Then, we compared the changes in gene expression before discharge (group 2) with pre-treatment (group 1), 2 months after SBRT treatment (group 3) with pre-treatment (group 1), and also group 3 vs group 2. Genes with $P<0.05$ were used as the differential gene, and the FPKM values of all differential genes in each comparison group were summarized. $\log_2$FC >0 (FC: fold change) was considered to be up-regulated, and $\log_2$FC <0 was considered to be down-regulated, the visualization of the heatmap of the liver cancer samples are shown in Supplementary Fig. 1. In addition, due to the excessive number of differentially expressed genes, we limited the criteria for differential genes. By using $q<0.05$ (q value is adjusted p value) and $|\log_2 FC| > 1$ as screening criteria, differentially expressed genes were found out. Then, we listed all the differentially expressed genes of group 2 vs 1, group 3 vs 1 and group 3 vs 2, it was found that a total of 16 differentially expressed genes remained elevated in all liver cancer patients after SBRT (i.e., up-regulated in group 2 vs 1 and in group 3 vs 1, but no difference in group 3 vs 2) (shown in Supplementary Fig.2A). In addition, a total of 12 differentially expressed genes remained downregulation in all liver cancers after SBRT (i.e. down-regulated in group 2 vs 1 and in group 3 vs 1, but no difference in group 3 vs 2) (shown in Supplementary Fig.2B).

Evaluation of the differential genes for SBRT efficacy in liver cancer

The expression of the above 28 genes were further verified by qPCR, which are shown in Supplementary Fig.3 A-C. Nonparametric test was used to determine whether there were differences in gene expression before and after SBRT. We found that 21 genes were differently expressed, the detailed results are shown in **Table 4**.

Furtherly, we calculated the changes of 21 genes and evaluated whether the change in group 2 vs 1, group 3 vs 1 were different between the effective and ineffective groups after SBRT. According to the imaging data of liver cancer patients, the therapeutic effects were divided into CR, PR, SD and PD based on the change of the diameter of liver cancer, CR and PR were classified into effective group, and SD and PD were classified into ineffective group. The results are shown in **Table 5**, it can be seen that ADIPOR1 and EPB42 were differentially expressed in pre-discharge versus before SBRT ($P<0.05$), indicating that ADIPOR1 and EPB42 had significant changes in the short term after SBRT treatment. Therefore, in the following study, we mainly focused on the two genes ADIPOR1 and EPB42.

Since there was no significant difference in group 3 vs 1, we only evaluated the prognostic value of the change ratio of group 2 vs 1 in the efficacy of 3-month after SBRT for liver cancer. The evaluation results of the prognostic value for ADIPOR1 and EPB42 were listed in **Table 6** and the ROC curves were shown in Supplementary Fig.4A-B. The results showed that ADIPOR1 had a sensitivity of 100% and a specificity of 83.33%, at the optimal threshold of 0.5838. And EPB42 had a sensitivity of 75% and a specificity of 100%, at the optimal threshold of 1.3817. Combined Table 5 with Table 6, it can be seen that when the change ratio of ADIPOR1 was lower than 0.5838 in pre-discharge compared with that before SBRT, patients had a better prognosis. Similarly, when the change of EPB42 was lower than 1.3817 in pre-discharge compared with that before SBRT, patients had a better prognosis after SBRT.

Discussion

In the current study, we mainly focused on evaluating the prognostic value of serum biomarkers, both the commonly used and novel biomarkers, for liver cancer patients treated by SBRT. Previous study reported that high AFP were associated with worse survival for liver patients who receiving Cyberknife treatment [15], however, all patients they included are in advanced or terminal stage of liver cancer. In our study, we evaluated the prognostic values of those existing tumor biomarkers, and found that those widely used tumor biomarkers such as AFP, CEA, CA199, etc. could not precisely predict the outcome of liver cancer treated by SBRT due to insufficient sensitivity or specificity, the Yoden index was less than 0.5. Actually, although AFP is a well-known biomarker, the use of AFP as a screening indicator for liver cancer has been cancelled by the 2010 American Association for the Study of Liver Diseases (AASLD) guidelines due to its low sensitivity[16]. So, it is necessary to find more effective biomarkers for accurate assessment of the efficacy of SBRT in patients with liver cancer. Since the development of liver cancer is accompanied by mRNA changes[17–19], whether the changes of mRNA in whole blood can indicate the prognosis remains to be studied.

Therefore, in our research, we explored potential biomarkers in mRNA level for the immediate-early assessment of prognosis after SBRT in patients with liver cancer through high-throughput sequencing and PCR. By using strict screening criteria, we finally got 28 differentially expressed mRNAs that were commonly increased or decreased after SBRT. Of all the 28 mRNAs, we found that the change ratio of ADIPOR1 (Z=-2.304, P = 0.021) and EPB42 (Z=-2.304, P = 0.021) in group 2 vs 1 was significantly different between the effective and ineffective groups at 3 months after SBRT. Adiponectin is a hormone produced by adipocytes, regulates metabolic processes. Adiponectin is known to bind 3 receptors: adiponectin receptor 1 (AdipoR1), adiponectin receptor 2 (AdipoR2), and T-cadherin[20]. Previous studies revealed that the expression of ADIPOR1 was significantly lower in liver cancer than non-neoplastic hepatic tissues[21], and low expression of ADIPOR1was associated with increased risk of recurrence and death in patients with liver cancer[22]. While the evidence suggests an inverse relation of ADIPOR1 to malignancy, another study pointed out that increase of ADIPOR1 correlated with cancer progression. Patients who had chronic hepatitis C with high serum adiponectin levels had a higher risk of liver cancer development [23]. In our study, ADIPOR1 is elevated after SBRT and had a good sensitivity and specificity in assessing the efficacy of SBRT after 3 months. Similarly, EPB42 (Erythrocyte membrane protein band 4.2) was also found to be a good prognostic biomarker for liver cancer treatment by SBRT. Furthermore, when the change of ADIPOR1 was lower than 0.5838 in pre-discharge compared with that before SBRT, patients had a better prognosis; when the change of EPB42 was lower than 1.3817 in pre-discharge compared with that before SBRT, patients had a better prognosis. In addition, routine blood-collection before treatment and before discharge, could not increase burden for patients, it is not only convenient to monitor the changes of ADIPOR1 or EPB42 and help to estimate whether the patient has a good prognosis, whether the patient needs further radiotherapy, consequently providing a reliable reference for the determination of the overall clinical treatment plan.

Although ADIPOR1 or EPB42 shown promising prognostic value for SBRT, it is still need more samples and further validation. In addition, how does ionizing radiation cause changes in ADIPOR1 and EPB42, and what's the underlying mechanism, remain to be studied.

Declarations

Ethical Approval and Consent to participate

The study design was approved by the Ethics Committee of the certain hospitals.

Consent for publication

The authors agree to publication.

Availability of data and material

The data used and analyzed in this study are available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests in regard to this study.

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Abbreviations

SBRT: Stereotactic body radiotherapy, CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease, OS: overall survival, DFS: disease-free survival, PFS: progression-free survival, EBRT: external beam radiation therapy, BED: Biologically Effective Dose, ROC: receiver-operating characteristic curve; AUC: Area under ROC

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Tables

Table 1. Analysis of the difference in the expression of tumor markers in patients before treatment, 3 months after treatment and 6 months after treatment

Tumor biomarker	3 months after SBRT vs before treatment					6 months after SBRT vs before treatment				
	Number*	Median level in 3 months after SBRT	Median level before treatment	Z	*P*	Number*	Median level in 6 months after SBRT	Median level Before treatment	Z	*P*
AFP	367	18.00 (5.92,182.20)	103.10 (7.86,1210.00)	-8.312	0.000	233	11.62(4.58,128.85)	52.51 (6.52,1176.00)	-6.141	0.000
CEA	309	2.65(1.77,4.21)	2.73(1.63, 4.55)	-0.888	0.375	204	2.83(1.85,3.98)	2.86(1.65,4.31)	-0.363	0.716
CA125	295	20.95(14.06, 59.95)	18.91(10.72, 44.12)	-2.997	0.003	197	21.24(12.42, 62.92)	16.56(9.80, 34.73)	-4.067	0.000
CA153	281	15.00(10.58,21.68)	14.57(10.22,21.29)	-0.540	0.589	193	13.38(9.33, 20.40)	13.59(9.74, 20.00)	-0.270	0.787
CA199	303	27.24(13.17,48.53)	26.40(12.01, 50.74)	-0.316	0.752	202	21.71(8.27, 44.24)	25.93(9.22, 50.05)	-2.551	0.011
CA724	285	1.42(1.05, 2.63)	1.30(0.98, 2.89)	-1.278	0.201	193	1.47(1.05, 2.69)	1.28(0.99, 2.72)	-1.204	0.229

* means the number of patients with an expression record before SBRT and 3 or 6 months after SBRT.

Table. 2 Analysis of the difference in the proportion of tumor markers in the survival group and the death group

Follow up time	Proportion of tumor markers	Survive number	Death number	proportion of tumor markers in survival group M (P_{25}, P_{75})	proportion of tumor markers in death group M (P_{25}, P_{75})	Z	*P*
One-year	AFP 3 months vs before	161	65	-0.318(-0.873, 0.276)	0.000(-0.557, 0.458)	-1.808	0.071
	AFP 6 months vs before	130	26	-0.380(-0.908,0.216)	0.000(-0.474, 0.860)	-2.264	0.024
	CA125 3 months vs before	123	49	0.085(-0.325, 0.580)	0.651(-0.217, 2.083)	-3.019	0.003
	CA125 6 months vs before	106	21	0.093(-0.358,0.733)	2.583(0.540, 13.327)	-4.056	0.000
	CA199 6 months vs before	108	22	-0.063(-0.367, 0.165)	0.333(-0.172,2.472)	-2.791	0.005
Two-year	AFP 3 months vs before	78	96	-0.446(-0.909, 0.272)	0.000(-0.593,0.471)	-2.313	0.021
	AFP 6 months vs before	65	51	-0.710(-0.928, 0.268)	0.000(-0.571, 0.716)	-2.934	0.003
	CA125 3 months vs before	59	71	0.051(-0.196, 0.459)	0.625(-0.273,1.991)	-2.864	0.004
	CA125 6 months vs before	53	39	-0.019(-0.451, 0.487)	0.959(-0.033, 5.315)	-3.504	0.000
	CA199 6 months vs before	56	40	-0.063(-0.360,0.164)	0.092(-1.312, 1.519)	-2.389	0.017
Three-year	AFP 3 months vs before	33	100	-0.349(-0.871, 0.468)	0.000(-0.635, 0.487)	-1.188	0.235
	AFP 6 months vs before	26	59	-0.750(-0.939, 0.609)	0.000(-0.574, 0.716)	-2.055	0.040
	CA125 3 months vs before	27	77	0.108(-0.316, 0.489)	0.651(-0.252, 1.787)	-2.324	0.020
	CA125 6 months vs before	21	46	0.029(-0.348,0.824)	0.851(-0.107, 4.753)	-2.365	0.018
	CA199 6 months vs before	22	47	0.045(-0.168, 0.305)	0.091(-0.308, 0.761)	-0.773	0.440

Table. 3 Evaluation of predictive efficacy in distinguishing survival group and death group of the tumor markers

	One year					Two-year					Three-year				
	Sensitivity	Specificity	Youden index	AUC	Cut-off	Sensitivity	Specificity	Youden index	AUC	Cut-off	Sensitivity	Specificity	Youden index	AUC	Cut-off
AFP 3 months vs before	——	——	——	——	——	46.2	77.1	0.23	0.600	≤-0.71	——	——	——	——	——
AFP 6 months vs before	40.8	92.3	0.33	0.641	≤-0.68	52.3	84.3	0.37	0.659	≤-0.68	53.8	81.4	0.35	0.640	≤-0.68
CA125 3 months vs before	79.7	51.0	0.31	0.648	≤0.63	86.4	52.1	0.39	0.647	≤0.55	88.9	53.2	0.42	0.651	≤0.54
CA125 6 months vs before	68.9	81.0	0.50	0.781	≤0.48	73.6	69.2	0.43	0.715	≤0.38	95.2	43.5	0.39	0.681	≤1.28
CA199 6 months vs before	80.6	59.1	0.40	0.689	≤0.23	89.3	37.5	0.27	0.642	≤0.35	——	——	——	——	——

AUC: Area under receiver-operating characteristic (ROC) curve.

Table 4. Gene expression levels before and after SBRT treatment

Gene	before discharge M (P_{25}, P_{75})	before SBRT M (P_{25}, P_{75})	*Z*	*P*	2 months after SBRT M (P_{25}, P_{75})	before SBRT M (P_{25}, P_{75})	*Z*	*P*
ADIPOR1	1.337(0.685, 2.419)	0.880(0.572, 2.630)	-2.722	0.006	2.245(0.931, 349.816)	1.059(0.606, 159.744)	-2.040	0.041
ANK1	0.003(0.001, 0.006)	0.004(0.001, 0.010)	-0.166	0.868	0.004(0.001, 3.542)	0.004(0.001, 0.013)	-0.784	0.433
ASCC2	0.042(0.015, 0.057)	0.03(0.015, 0.066)	-1.207	0.227	0.047(0.019, 1.012)	0.072(0.019, 2.103)	-2.275	0.023
BCAM	0.000(0.000, 0.002)	0.000(0.000,0.002)	-1.065	0.287	0.001(0.001,1.040)	0.000(0.000,0.003)	-1.647	0.099
BCL11B	0.004(0.003, 0.011)	0.009(0.006, 0.024)	-3.195	0.001	0.015(0.011, 0.787)	0.011(0.006, 2.129)	-0.471	0.638
BCL2L1	0.503(0.271,0.965)	0.581(0.237,1.207)	-1.965	0.049	0.731(0.303,35.545)	0.738(0.334, 15.770)	-1.334	0.182
BLK	0.001(0001, 0.016)	0.003(0.002,0.010)	-2.296	0.022	0.004(0.001, 0.206)	0.003(0.002, 1.517)	-1.647	0.099
BTLA	0.029(0.017, 0.079)	0.045(0.023, 0.082)	-1.586	0.113	0.060(0.024, 0.880)	0.044(0.023, 3.730)	-0.941	0.347
CAT	0.117(0.074, 0.220)	0.154(0.104, 0.304)	-2.817	0.005	0.204(0.133, 3.892)	0.175(0.116, 7.493)	-1.098	0.272
CD79A	0.016(0.010,0.050)	0.046(0.025, 0.118)	-3.575	0.000	0.053(0.012, 3.221)	0.068(0.028, 7.370)	-1.962	0.050
COL19A	0.004(0.001, 0.021)	0.013(0.006, 0.087)	-3.243	0.001	0.013(0.002, 0.097)	0.013(0.004, 1.018)	-1.412	0.158
CXCR5	0.001(0.000, 0.003)	0.003(0.001, 0.004)	-3.527	0.000	0.002(0.000, 0.175)	0.003(0.002, 1.550)	-1.726	0.084
EPB42	0.039(0.015, 0.068)	0.027(0.011, 0.054)	-2.817	0.005	*0.058(0.021, 3.540)*	*0.026(0.011, 1.543)*	-3.059	0.002
GOLGA6L9	0.001(0.000, 0.004)	0.002(0.000, 0.006)	-2.154	0.031	0.003(0.000, 0.052)	0.004(0.001, 0.316)	-2.197	0.028
IL7R	0.176(0.113, 0.602)	0.492(0.292, 1.024)	-3.385	0.001	0.536(0.265, 17.622)	0.665(0.274, 53.358)	-1.726	0.084
KLHL14	0.000(0.000, 0.002)	0.001(0.001, 0.007)	-3.385	0.001	0.001(0.000, 0.049)	0.002(0.000, 1.031)	-2.118	0.034
MAP2K3	0.053(0.027,0.085)	0.044(0.029, 0.088)	-1.538	0.124	0.060(0.024, 57.292)	0.070(0.031, 7.327)	-1.020	0.308
OR2W3	0.008(0.004, 0.020)	0.008(0.003, 0.017)	-1.349	0.177	0.010(0.007, 29.623)	0.010(0.004, 9.895)	-2.353	0.019
OSBP2	0.000(0.000, 0.001)	0.000(0.000, 0.001)	-1.870	0.061	0.000(0.000, 9.563)	0.000(0.000, 4.499)	-1.256	0.209
PAX5	0.001(0.000, 0.003)	0.003(0.003, 0.006)	-3.479	0.001	0.003(0.001, 0.279)	0.004(0.002, 1.416)	-2.197	0.028
FECH	0.396(0.178, 0.732)	0.355(0.159, 0.655)	-1.728	0.084	0.597(0.440, 2.363)	0.468(0.223, 0.997)	-2.197	0.028
PIM1	0.241(0.160, 0.435)	0.176(0.073, 0.398)	-1.870	0.062	0.397(0.149,1.296)	0.242(0.065, 0.493)	-1.334	0.182
SFRP2	0.002(0.001, 0.007)	0.002(0.001, 0.007)	-2.107	0.035	0.002(0.001, 0.821)	0.003(0.001, 0.215)	-1.020	0.308
STAP1	0.005(0.002, 0.014)	0.016(0.007, 0.030)	-3.574	0.000	0.016(0.006, 0.156)	0.019(0.007, 1.553)	-1.647	0.099
TMCC2	0.025(0.014, 0.080)	0.014(0.010, 0.116)	-2.249	0.025	0.044(0.018, 7.788)	0.025(0.011, 1.927)	-1.961	0.050
UBA52	6.383(4.086, 16.038)	5.160(3.247, 21.536)	-0.970	0.332	12.730(7.651, 173.134)	6.438(3.963, 79.486)	-2.118	0.034
UBB	9.989(3.795, 13.428)	4.941(2.943, 8.312)	-1.491	0.136	9.453(7.354, 19.715)	4.937(3.143, 8.559)	-2.275	0.023
YBX1	0.866(0.531, 1.797)	1.188(0.452, 1.936)	-0.876	0.381	1.865(0.529, 12.223)	1.348(0.714, 4.095)	-1.412	0.158

Table 5. Change ratio of differentially expressed genes in the effective and ineffective groups of liver cancer at 3 months after SBRT

Differential gene	Change ratio of the expression before discharge and before SBRT (2vs1)				Change ratio of the expression in 2 months after SBRT and before SBRT (3vs1)			
	Effective	Ineffective	*Z*	*P*	Effective	Ineffective	*Z*	*P*
ADIPOR1	0.205(-0.112, 0.571)	1.020(0.874, 1.484)	-2.304	0.021	0.464 (-0.098, 1.400)	1.298(0.281, --)	-1.202	0.229
ASCC2	0.149(-0.218, 0.479)	0.675(-0.034, 1.591)	-1.455	0.146	0.376(-0.080, 1.503)	0.315(0.276, --)	-0.277	0.782
BCL11B	-0.514(-0.820, -0.127)	-0.523(-0.706, -0.508)	-0.364	0.716	0.670(-0.591, 1.578)	-0.473(-0.898, --)	-1.387	0.166
BCL2L1	0.307(0.039, 0.865)	0.196(-0.285, 0.989)	-0.364	0.716	0.546(0.005, 1.502)	-0.342(-0.619, --)	-0.647	0.518
BLK	-0.512(-0.864, 0.024)	-0.496(-0.847, -0.325)	-0.485	0.628	-0.523(-0.842, 1.522)	-0.433(-0.926, --)	-0.277	0.782
CAT	-0.454(-0.624, 0.040)	-0.176(-0.669, 0.194)	-0.485	0.628	-0.369(-0.509, 0.630)	0.310(-0.740, --)	-0.277	0.782
CD79A	-0.817(-0.892, -0.507)	-0.592(-0.849, -0.306)	-0.789	0.430	-0.564(-0.662, 0.408)	-0.564(-0.680, --)	-0.185	0.853
COL19A	-0.823(-0.918, -0.354)	-0.384(-0.889, 0.030)	-0.606	0.544	-0.608(-0.853, 0.685)	-0.221(-0.933, --)	-0.092	0.926
CXCR5	-0.628(-0.876, -0.357)	-0.594(-0.900, -0.080)	-0.121	0.903	-0.495(-0.763, 0.516)	-0.609(-0.912, --)	-0.647	0.518
EPB42	0.400(0.087, 0.589)	2.062(0.747, 2.678)	-2.304	0.021	1.303(0.537, 1.772)	1.616(1.538, --)	-1.757	0.079
GOLGA6L9	-0.440(-0.811, -0.088)	-0.757(-0.869, 0.526)	-0.728	0.467	-0.227(-0.656, 0.093)	-0.710(-0.860, --)	-1.757	0.079
IL7R	-0.721(-0.828, -0.514)	-0.595(-0.726, -0.014)	-1.213	0.225	-0.222(-0.672, 0.955)	-0.188(-0.673, 0.266)	-0.092	0.926
KLHL14	-0.846(-0.900, -0.566)	-0.103(-0.901, 1.542)	-1.455	0.146	-0.535(-0.792, -0.003)	-0.346(-0.955, 1.576)	-0.462	0.644
OR2W3	-0.041(-0.150, 1.198)	0.236(0.077, 1.56)	-1.091	0.275	0.886(0.156, 1.424)	0.316(-0.364, 2.293)	-0.462	0.644
PAX5	-0.777(-0.927, 0.253)	-0.894(-0.919, -0.547)	-0.243	0.808	-0.561(-0.855, 0.088)	-0.716(-0.888, -0.651)	-1.202	0.229
FECH	0.117(-0.175, 1.496)	0.496(0.218, 1.053)	-0.970	0.332	0.693(0.146, 1.630)	0.313(-0.362, --)	-0.092	0.926
SFRP2	0.408 (-0.083, 1.400)	0.482(-0.03, 3.984)	-0.849	0.396	0.178(-0.248, 1.267)	0.318(-0.629, 1.702)	-0.277	0.782
STAP1	-0.737(-0.862, -0.539)	-0.760(-0.918, -0.469)	-0.485	0.628	-0.344 (-0.671, 0.656)	-0.406(-0.919, 0.075)	-0.647	0.518
TMCC2	0.407(-0.091, 0.615)	0.961(-0.015, 1.467)	-1.334	0.182	1.441(0.128, 2.334)	0.269(-0.257, 1.577)	-0.832	0.405
UBA52	-0.003 (-0.281, 0.679)	0.124(-0.067,1.043)	-1.213	0.225	1.244(-0.096,1.829)	0.307(0.03, 2.915)	-0.092	0.926
UBB	-0.085(-0.238, 0.578)	0.591(0.51,2.296)	-1.940	0.052	1.135 (0.014,2.219)	1.295(0.36, 2.302)	-0.462	0.644

Table 6. The evaluation results of the prognostic value of the change ratio of ADIPOR1 and EPB42 (2vs1) for liver cancer patients in 3 months after SBRT

mRNA	AUC	SE	Sensitivity	Specificity	Yoden index	Cut-off
			□%□	□%□		
ADIPOR1	0.896	0.087	100	83.33	0.83	0.5838
EPB42	0.896	0.111	75.00	100.00	0.75	1.3817

*2vs1, means pre-discharge versus before SBRT

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