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A system for tumor heterogeneity evaluation and diagnosis based on tumor markers measured routinely in the laboratory

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ABSTRACT

Objectives: To develop an efficient and reliable approach to estimate tumor heterogeneity and improve tumor diagnosis using multiple tumor markers measured routinely in the clinical laboratory.

Methods: A total of 161 patients with different cancers were recruited as the cancer group, and 91 patients with non-oncological conditions were required as the non-oncological disease group. The control group comprised 90 randomly selected healthy subjects. AFP, CEA, CYFRA, CA125, CA153, CA199, CA724, and NSE levels were measured in all these subjects with a chemiluminescent microparticle immunoassay. The tumor marker with the maximum S/CO value (sample test value:cutoff value for discriminating individuals with and without tumors) was considered as a specific tumor marker (STM) for an individual. Tumor heterogeneity index (THI) = N/P (N: number of STMs; P: percentage of individuals with STMs in a certain tumor population) was used to quantify tumor heterogeneity: high THI indicated high tumor heterogeneity. The tumor marker index (TMI), TMI = STM × (number of positive tumor markers + 1), was used for diagnosis.

Results: The THIs of lung, gastric, and liver cancers were 8.33, 9.63, and 5.2, respectively, while the ROC-areas under the curve for TMI were 0.862, 0.809, and 0.966.

Conclusion: In this study, we developed a novel index for tumor heterogeneity based on the expression of various routinely evaluated serum tumor markers. Development of an evaluation system for tumor heterogeneity on the basis of this index could provide an effective diagnostic tool for some cancers.

1. Introduction

Tumor heterogeneity, a well-known concept in tumor biology, refers to biological differences among cells within a single tumor. Since this phenomenon is significantly responsible for difficulties in tumor diagnosis and therapy, the detailed description of tumor heterogeneity and classification and observation of tumors at the molecular level may substantially aid in these two processes.

Tumor heterogeneity is generally attributed to intrinsic genetic insvility [1–3]. Therefore, the diagnosis of any one type of tumor cannot be based on a single tumor marker. With long-term observations and examinations, an increasing number of proven serum tumor markers have been used in the clinic, and detection approaches and effective positive criteria for common serum tumor markers are well established including alpha fetoprotein (AFP) for hepatocellular carcinoma [4–6], carcinoembryonic antigen (CEA) for cancers in digestive tract [7–9], CYFRA 21-1 for breast carcinoma [10–12], CA125 [13–15] for ovarian cancer, CA15-3 for breast cancer [16–18], CA19-9 for pancreatic cancer [19–21], CA72-4 for ovarian cancer [22–24], and neuron specific enolase (NSE) for lung cancer [25–27]. We hypothesized that serum tumor markers would be useful for establishing an evaluation system for tumor heterogeneity and for diagnosis of some types of tumors. We selected tumor markers that are routinely measured in the laboratory for analysis since reliable assays for these markers are commercially available. Our results may form the basis for an efficient and reliable approach to estimate heterogeneity and study tumor biology for better tumor diagnosis and therapy.

2. Methods

2.1. Subjects

A total of 161 patients (98 men and 63 women; mean age, 60.5 ± 10.8 years) with selected cancers (61 had gastric cancer; 50, liver cancer; and 50, lung cancer) in stage I or over stage I by TNM staging classification were recruited as the cancer group; these cancer patients were evidenced by surgical intervention and pathologic findings (most of gastric cancers, 55/61, were adenocarcinoma; most of lung cancers, 43/50, were non–small-cell lung cancer and all of liver cancers were hepatocellular carcinoma). The samples were collected before surgery without receiving any drug treatment.

Abbreviations: AUC, area under the curve; NPM, number of positive tumor markers; ROC, receiver operating characteristic; S/CO, signal-to-cutoff ratio; STM, Specific tumor marker; THI, tumor heterogeneity index; TMI, tumor marker index.

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Table 1
The raw data of various tumor markers in the three different tumors, other diseases and healthy subjects (Mean ± SD).

Biomarker (Units)	Cancer groups			Other diseases	Normal control	*P*
	Lung	Gastric	Liver			
AFP(ng/ml)	4.3 ± 7.8	13.4 ± 77.3	4110.4 ± 17,280.5	2.4 ± 1.4	2.3 ± 1.2	<0.001
CEA(ng/ml)	10.8 ± 22.6	35.4 ± 90.3	21.8 ± 70.8	3.5 ± 6.4	1.6 ± 0.8	<0.001
CYFRA(ng/ml)	3.6 ± 2.0	3.8 ± 5.7	13.6 ± 43.0	2.1 ± 1.1	1.8 ± 0.8	<0.001
CA125(IU/ml)	67.5 ± 218.5	17.3 ± 22.4	70.0 ± 107.2	6.7 ± 6.3	10.7 ± 5.0	<0.001
CA153(IU/ml)	23.8 ± 34.2	15.5 ± 12.1	17.4 ± 15.1	10.4 ± 6.7	12.2 ± 6.2	<0.001
CA199(IU/ml)	55.4 ± 170.2	36.3 ± 129.4	439.4 ± 2005.6	11.6 ± 7.0	11.6 ± 6.2	<0.001
CA724(IU/ml)	9.1 ± 25.3	9.1 ± 16.5	8.9 ± 20.3	4.2 ± 4.6	1.9 ± 1.3	<0.001
NSE(IU/ml)	17.8 ± 18.9	10.3 ± 8.9	20.3 ± 29.1	10.5 ± 12.5	9.3 ± 3.8	<0.001

The *P* value is for the comparison of tumor marker versus control.

Then, 91 patients (51 men and 40 women; mean age, 61.5 ± 15.7 years) with non-oncological conditions were recruited as other disease group: 70 had cerebrovascular disease and 20 had fractures. The control group comprised 90 randomly selected healthy subjects (56 men and 34 women; mean age, 60.2 ± 11.3 years).

All individuals were northern Han Chinese residing in China. All of the experiments on the subjects were conducted in accordance with the Declaration of Helsinki, and the study was approved by the Institutional Ethics Committee of Dalian Medical University.

2.2. Measurement of tumor markers

The following tumor markers routinely measured in at our clinical laboratory were selected: AFP (0–7.0 ng/ml), CEA (0–4.7 ng/ml), CYFRA 21-1 (0–3.3 ng/ml), CA125 (0–35 IU/ml), CA15-3 (0–25 IU/ml), CA19-9 (0–27 IU/ml), CA72-4 (0–6.9 IU/ml) and NSE (0–16.3 IU/ml). These 8 tumor markers were measured in the serum of all study participants using a chemiluminescent microparticle immunoassay with the ROCHE E411 device (F. Hoffmann-La Roche, Ltd., Switzerland) and standard commercial reagent kits. Briefly, sample (tumor marker) and anti-tumor marker-coated paramagnetic microparticles are combined. tumor marker present in the sample binds to the anti-tumor marker-coated microparticles. After washing, acridinium-labeled anti-tumor marker conjugate is added in the next step. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured in relative light units (RLUs). A direct relationship exists between the amount of tumor marker in the sample and the RLUs detected. The amount of a tumor marker can be obtained from standard substance. The chemiluminescent immunoassay is a highly sensitive screening assay for detecting tumor marker [28–30]. The significance of the differences was determined by Kruskal–Wallis H tests for abnormally distributed continuous data. Data were considered to be significant if the probability of a type I error was < 0.05. Calculations were carried out using SPSS software for Windows (SPSS, Chicago, IL, USA).

2.3. Specific tumor markers

It was necessary to re-screen these markers to exclude some tumor markers expressed at high levels in control group for keeping specificity of tumor markers in a new system. First, the distribution of tumor markers in patients with non-oncologic conditions was examined. If markers with a positive rate exceeding 5% in control group, these markers were considered invalid and excluded. The remaining markers were considered valid and included in the construction of the tumor heterogeneity evaluation system.

The ratio between the sample test value and the cut-off value was defined as the S/CO value for comparing measured results between different tumor markers. For individuals with tumors, multiple S/CO values were obtained when several tumor markers were simultaneously detected, and the S/CO values of different tumor markers were compared. The higher the S/CO value was, the higher the expression level of the tumor marker was. Therefore, the marker with the maximum S/CO value was considered as a specific tumor marker (STM) for the individual. Theoretically, each cancer patient could have only one STM.

2.4. Quantification of tumor heterogeneity

For a certain tumors, such as lung cancer and liver cancer, there were multiple STMs: the greater the number of STMs was, the greater would the tumor heterogeneity be.

In some cases, none of the selected markers tested positive in a particular tumor patient, because of which tumor heterogeneity evaluation on the basis of STM was difficult. To solve this problem, we drew on the concept of tumor heterogeneous index (THI), whereby the tumor heterogeneity of a positive population in a certain tumor population is considered to represent the heterogeneity in the entire tumor population:

$$THI = N/P.$$

N: number of STMs; P: percentage of individuals with STMs in a certain tumor population.

For instance, suppose there were 60 patients with at least one tumor markers in 100 patients with a certain tumor and the number of STMs in these 60 tumor patients was 4, then the THI would be 6.7 (4/0.6).

The THI can also mean that 100% tumor could be marked by using the number of STMs for a certain type tumor, thus, 7 tumor markers would be adequate to confirm the diagnosis of the above tumor. Another interpretation of this result is that this tumor has 7 biological categories.

The higher the THI was, the higher the tumor heterogeneity would be. If one tumor marker could indicate tumor of 100%, the heterogeneity of this tumor was 1.0. This indicates that tumors with THI = 1 had no heterogeneity for this tumor.

2.5. Establishment of tumor diagnosis system

Both the S/CO value of the STM and the number of positive tumor markers (the number of positive tumor markers was proportional to THI) for a tumor patient needed to be considered during establishment

Table 2
Positive rate (%) of various tumor markers in the three different tumors, other diseases and healthy subjects.

Biomarker	Lung cancer	Gastric cancer	Liver cancer	Other diseases	Normal control
AFP	4.0	6.6	78.0	1.1	1.1
CEA	32.0	44.3	26.0	5.5	1.1
CYFRA	48.0	11.5	44.0	5.5	5.6
CA125	28.0	6.6	42.0	1.1	0.0
CA153	16.0	14.8	8.0	3.3	5.6
CA199	18.0	13.1	60.0	0.0	0.0
CA724	18.0	31.1	14.0	14.3	0.0
NSE	22.0	19.7	36.0	18.7	4.4

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