

GENE SIGNATURES FOR USE WITH HEPATOCELLULAR CARCINOMA

FIELD OF THE INVENTION

5 The present invention relates to a method of analyzing hepatocellular carcinoma (HCC) patients using the expression levels of various immune genes in a sample from the tumor, in particular to predict the prognosis of HCC patients. The invention also relates to methods of identifying an agent effective for treating HCC, and also to methods for stratifying HCC.

10 All documents cited in this text ("herein cited documents") and all documents cited or referenced in herein cited documents are incorporated by reference in their entirety for all purposes. There is no admission that any of the various documents etc. cited in this text are prior art as to the present invention.

BACKGROUND

15 Hepatocellular carcinoma is an aggressive malignancy and claims over 600,000 lives every year worldwide. HCC incidence is rising in Western countries partly due to increased Hepatitis C virus (HCV) infection. HCC is a heterogeneous disease comprising distinct molecular and clinical subgroups [5-6]. This is largely due to the different HCC etiologies which include hepatitis, alcohol and non-alcohol induced cirrhosis. Geographical and ethnic variations further contribute to its heterogeneity.

20 There are few treatment options for HCC, in particular for patients with advanced disease where there are limited treatments. Resection remains the treatment choice for many patients but it is also associated with high relapse rate and poor 5-year survival rate. Sorafenib, a tyrosine kinase inhibitor recently approved for advanced HCC, brings only limited improvement in survival [3]. More aggressive treatments, including liver transplantation for
25 suitable patients, improves survival. However, identifying HCC patients likely to benefit from such approaches remains challenging.

30 With the development of health awareness in the general public, HCC comes to medical attention at earlier stages where often it is hard to determine the prognosis using classical histopathological measurements such as tumor multinodularity and vascular invasion. In the past decade, several laboratories used gene-expression profiling to define the molecular nature and identify prognostic signatures for HCC [8-12]. However, little consensus was reached from such efforts, illustrating the complexity and heterogeneity of this cancer. Each study focused on different molecular pathways and limited attention has so far been given to the tumor immune microenvironment.

35 SUMMARY OF THE INVENTION

In one aspect, there is provided a method of analysing a patient with Hepatocellular Carcinoma (HCC), wherein the method comprises: (a) detecting the expression levels of 5, 8, 7, 11, or 14 of genes selected from the group consisting of CCL5, CCR2, CEACAM8, CXCL10, IFNG, IL6, NCR3, TBX21, TLR3, TNF, CCL2, CD8A, FCGR1A, LTA, and TLR4
40 in a patient-derived tumor sample; and (b) based on a gene signature as listed in Table 2A, Table 2B, Table 3, Table 4, Table 14, Table 15 and/or Table 16, determine one or more of the following: stratifying or classifying the patient to have HCC; providing a prognosis for the patient with HCC; monitoring disease progression of HCC; predicting efficacy of a therapeutic

intervention, selecting treatment for the tumor, or evaluating the efficacy of a therapeutic intervention for a patient with HCC.

In another aspect, there is provided the use of reagents for determining the expression of said 5, 8, 7, 11, or 14 of genes selected from the group consisting of CCL5, CCR2, CEACAM8, CXCL10, IFNG, IL6, NCR3, TBX21, TLR3, TNF, CCL2, CD8A, FCGR1A, LTA, and TLR4 in a method of the present invention.

In yet another aspect, there is provided a computer program or computer software product for performing step (b) of a method of the present invention, or a computer system programmed to perform step (b) of a method of the present invention.

In a further aspect, there is provided the use of a microarray comprising a plurality of probes capable of hybridizing to the 5, 8, 7, 11, or 14 of genes selected from the group consisting of CCL5, CCR2, CEACAM8, CXCL10, IFNG, IL6, NCR3, TBX21, TLR3, TNF, CCL2, CD8A, FCGR1A, LTA, and TLR4 in a method of the present invention.

In another aspect, there is provided a method of providing a an HCC human patient with a good or a poor prognosis, wherein the method comprises: (a) determining the expression levels of 5, 8, 7, 11, or 14 of genes selected from the group consisting of CCL5, CCR2, CEACAM8, CXCL10, IFNG, IL6, NCR3, TBX21, TLR3, TNF, CCL2, CD8A, FCGR1A, LTA, and TLR4 in a tumor sample derived from said patient, which tumor sample comprises total tumor material, (b) analysing the genes based on the gene signatures as listed in table Table 2A, Table 2B, Table 3, Table 4, Table 14, Table 15 and/or Table 16, ; and (b) determining the similarity of an expression profile comprising the expression levels determined in step (a) to a good prognosis template which comprises gene expression levels characteristic of good prognosis patients and a poor prognosis template which comprises gene expression levels characteristic of poor prognosis patient, wherein a higher similarity of said expression profile to said good prognosis template indicates a poor prognosis and a higher similarity to said poor prognosis template than to said good prognosis template indicates a poor prognosis, and wherein a poor prognosis is less than the median survival years of a given cohort and a good prognosis is more than the median survival years of a given cohort.

The current disclosure alsoinvention describes an immune gene signature derived from resected HCC tumors from Singapore HCC patients (n=61) who are mostly at stage I, for predicting prognosis or survival in HCC patients. The immune gene signature has been

validated as being able to predict survival of HCC patients from another region in Asia, Hong Kong (n=56) as well as from Europe, Zurich, Switzerland (n=55); both the Hong Kong and Zurich cohort include more advanced HCC patients- mostly Stage II or III.

- 5 | In ~~at least some examples~~ ~~embodiments~~, the gene signature includes a combination of three to fifteen (and preferably five to fourteen) immune genes out of a total 15 immune genes of interest whose relative expression is preferably analysed in a classifier (algorithm). Overall, an increase in mRNA expression of these genes is associated with better prognosis. The predictive power of the combination of any five to fourteen immune genes (the classifier) of
- 10 | these 15 immune genes is stronger than any single individual gene by itself.

This immune signature can be used by itself to analyse HCC patients or with other information, such as staging information. This application describes various uses of the immune signature, in particular in predicting the prognosis of HCC patients (e.g. $<$ of 5 years survival).

~~In one example, there is a preferred embodiment of the invention~~ provides a method for predicting prognosis ($<$ 5 years survival) of hepatocellular carcinoma patients based on measurement of the relative level of expression of a combination of three to fifteen immune genes (and preferably 5 to 14 immune genes) out of 15 immune genes of interest in the tumors of such patients. Tumor material can come from surgical resection or biopsy. The relative gene expression information can optionally be combined in an algorithm.

GLOSSARY OF TERMS

This section is intended to provide guidance on the interpretation of the words and phrases set forth below (and where appropriate grammatical variants thereof). Further guidance on the interpretation of certain words and phrases as used herein (and where appropriate grammatical variants thereof) may additionally be found in other sections of this specification.

As used herein, the singular form "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "an agent" includes a plurality of agents, including mixtures thereof and reference to "the nucleic acid sequence" generally includes reference to one or more nucleic acid sequences and equivalents thereof known to those skilled in the art, and so forth.

As used herein, the term "comprising" means "including". Thus, for example, a gene signature "comprising three genes" may consist exclusively of three genes or may include one or more further marker genes.

The term "stratifying" as used herein refers to describing or separating a patient population into more homogeneous subpopulations according to specified criteria. In one embodiment, patients can be stratified for different treatment protocols (e.g. more or less aggressive treatment, surgical intervention, liver transplantation, immunotherapy, chemotherapy with a given drug or drug combination, and/or radiation therapy). Patients may also be stratified into those having a poor or good prognosis, or those have a short or long predicted survival.

The term "classifying" as used herein refers to the process of determining or arranging patients into a particular group depending on their tumor sample profile. In at least some embodiments, the term "classifying" refers to classifying a patient as having a particular prognosis, e.g. a poor or good prognosis, or short or long predicted survival.

The term "prognosis" as used herein relates to providing a forecast or prediction of the likely course or outcome of HCC. The term includes a reference to predicting HCC progression (e.g. recurrence or metastatic spread), survival, drug resistance, partial or complete remission, or a good or poor outcome (good or poor prognosis respectively). The term also includes predicting the timing of any of the aforementioned (e.g. more than, less than, or equal to a given number of years (e.g. 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more years)). Thus, for instance, providing a prognosis may comprise predicting the patient's survival as being more than, less than, or equal to a given number of years.

Where survival, recurrence, metastatic spread or another event is described herein in relation to a given period of time, the period of survival may optionally be measured from first