Biomarkers and Personalized Sorafenib Therapy

Compared with other types of cancer, hepatocellular carcinoma (HCC) is highly heterogeneous because of the intrinsic diversity in its pathogenesis, molecular heterogeneity, its multicentric occurrence, and its etiology, among others. Until now, locoregional therapies such as resection, ablation, and transarterial chemoembolization (TACE) have been performed extensively to treat HCC because the application of systemic chemotherapy using cytotoxic anticancer drugs is limited by the presence of pancytopenia and hepatic dysfunction caused by the cirrhosis that usually accompanies HCC. Since its approval in 2007 as a systemic chemotherapeutic agent that improves the survival of patients with unresectable advanced HCC, sorafenib has been widely used as a novel treatment option in patients with advanced HCCs with extrahepatic spread and/or vascular invasion.

Need for Personalized Sorafenib Therapy

Sorafenib is the only anticancer drug with proven prognostic efficacy in HCC [1, 2]; however, it should be administered with care because of various adverse effects, including hand–foot skin reaction, hypertension, diarrhea, and liver dysfunction. Moreover, because cases of ineffective and incomplete response to sorafenib have been reported, and because the drug is extremely expensive, the development of prognostic factors and biomarkers for predicting adverse events are eagerly awaited. The ability to predict treatment outcome and adverse events would make it possible to avoid administering sorafenib to patients who would not benefit from it and to exclude patients likely to develop serious side effects. This would not only increase safety but also markedly improve the medico-economic situation. Consequently, studies are currently underway to develop biomarkers for predicting treatment outcome and adverse events, and some biomarkers for novel molecularly targeted drugs have already been identified in the early phase of clinical trials and subsequently evaluated in phase III trials.
Signals Triggering Proliferation of Hepatocellular Carcinoma

Mutations of specific genes that play an important role in cancer proliferation (oncogene addiction), such as the BRAF V600E mutation in malignant melanoma [3] and the ALK fusion gene mutation in lung cancer [4], are perfect targets for molecularly targeted drugs. However, with regard to HCC, no study has reported a specific gene mutation that causes oncogene addiction, despite various proliferation-related signal transduction pathways being activated, such as those of MAP kinase [5], PI3K/Akt/mTOR [6], c-MET [7], IGF [8], Wnt-beta-catenin [9, 10], hedgehog [11], vascular endothelial growth factor (VEGF) receptor, and platelet-derived growth factor (PDGF) receptor [12]. It is therefore difficult to use gene mutations as biomarkers in HCC. The multikinase inhibitor sorafenib suppresses the proliferation of vascular endothelial cells and pericytes by inhibiting the activity of tyrosine kinase in the cytosolic domain of the VEGF and PDGF receptors. It also suppresses the proliferation of tumor cells by inhibiting the activity of Raf serine/threonine kinase in the MAP kinase cascade responsible for such proliferation. Sorafenib, therefore, exerts a multifaceted anticancer effect by inhibiting various kinases and appears to work well in HCC. In other words, the difficulty in identifying a specific biomarker in sorafenib therapy for HCC may be caused by the presence of multiple molecular targets. Despite previous reports of potential biomarkers, no definitive biomarker for sorafenib has been reported (Table 1 [13–24]).

FGF3/4 Gene Amplification

The SHARP [1] and Asia Pacific [2] trials revealed extremely low response rates in patients treated with sorafenib: the overall response was as low as a few percent, with almost no tumor response. In general, a drug's inhibitory action on tumor proliferation improves patient survival. In Japan, there has been a stream of super responders (i.e., complete responders and partial responders) to sorafenib since its approval in 2009, suggesting a common, yet unknown response factor in Japanese people [25]. In fact, when we gathered biopsy specimens from sorafenib super responders across Japan and performed genome analysis, the results revealed a high expression level of FGF3/4, lung metastasis, and poorly differentiated HCC as common factors [24], suggesting that each of these factors could serve as biomarkers. Although high expression of FGF3/4 may be a good marker because of its affinity for various molecular targets, it is difficult to use high levels of FGF3/4 expression as a general purpose marker because the FGF3/4 mutation has been observed in only a few percent of all HCC patients.

c-Jun N-Terminal Kinase

In addition, c-Jun N-terminal kinase (JNK), which acts as an intracellular signaling protein, is highly upregulated in patients with no response to sorafenib. Because JNK activation is closely related to CD133 expression, CD133 and JNK expression may serve as a biomarker for no tumor response to sorafenib [23].
Miyahara et al. measured changes in the levels of several serum angiogenesis markers before and after sorafenib treatment and showed that the number of HCC patients with progressive disease was high among HCC patients with high levels of serum angiogenesis markers before the treatment [20]. In addition, Tsuchiya et al. reported that chronological changes in serum VEGF levels can serve as a useful prognostic indicator [19]. As shown in these studies, changes in the levels of serum markers during drug therapy can be used to predict treatment outcome to a certain extent.

Although the aim is to use biomarkers to predict treatment outcome and adverse events before drug treatment, this is difficult to do at present. We await the results of genome-wide association studies currently underway. Another way to predict adverse events is to monitor laboratory test values and hemodynamics throughout drug therapy.

### Table 1. Potential biomarkers for predicting treatment outcome and adverse events of sorafenib treatment in HCC

| Biomarker                                      | Author       | Year | Reference |
|------------------------------------------------|--------------|------|-----------|
| Symptoms and signs                             |              |      |           |
| Dermal toxicity                                | Vincenzi B   | 2010 | 13        |
| Hypertension                                   | Estfan B     | 2013 | 14        |
| Lung metastasis                                | Yau T        | 2009 | 21        |
| Serum markers                                  |              |      |           |
| AFP decrease after 6 weeks (>20%)              | Yau T        | 2011 | 15        |
| AFP decrease in the early phase (comparing AFP after 2 and 4 weeks) | Kuzuya T | 2011 | 16 |
| DCP increase after 2 weeks                     | Ueshima K    | 2011 | 17        |
| NX-DCP and DCP                                | Miyahara K   | 2013 | 18        |
| Serum VEGF decrease after 8 weeks              | Tsuchiya K   | 2014 | 19        |
| Angiopoietin-2, G-CSF, HGF, leptin             | Miyahara K   | 2011 | 20        |
| Genes and proteins                             |              |      |           |
| pERK                                           | Zhang Z      | 2009 | 22        |
| JNK activity                                   | Hagiwara S   | 2012 | 23        |
| FGF3/FGF4 amplification                        | Arao T       | 2013 | 24        |

AFP = alpha-fetoprotein; DCP = des-γ-carboxyprothrombin; G-CSF = granulocyte colony-stimulating factor; HGF = hepatocyte growth factor; pERK = phosphorylated extracellular-signal-regulated kinases.

### Cytokine Biomarkers

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Proteins Induced by Vitamin K Absence

Proteins induced by vitamin K absence (PIVKA)-II are markers for HCC. In hepatic cells, γ-glutamyl carboxylase and vitamin K are coenzymes responsible for the carboxylation of ten glutamic acid residues in the Gla domain located at the N-terminus of the prothrombin precursor, thereby converting the precursor to prothrombin, which possesses coagulation activity and is secreted into the bloodstream. However, when vitamin K is deficient, prothrombin is released into the circulation without complete carboxylation. PIVKA-II proteins are the non-functional precursors that accumulate in the circulation. Because sorafenib administration rapidly increases PIVKA-II levels in many cases, we retrospectively investigated the relationship between tumor progression and increases in PIVKA-II levels. The results showed that time to progression (TTP) was significantly prolonged in patients whose PIVKA-II level had been upregulated two-fold 2 weeks after sorafenib administration [17]. We believe that prolonged TTP is a reflection of ischemia in HCC caused by the anti-angiogenic effect of sorafenib. Such ischemia alters the actin molecules making up the cytoskeleton of HCC cells, thereby impairing the endocytosis of vitamin K. This subsequently leads to vitamin K deficiency and an increase in PIVKA-II levels, and means that PIVKA-II has potential as a surrogate marker for cellular ischemia as well as a monitoring marker for the anti-angiogenic effect of sorafenib.

Comparison of Images Taken Before and after Treatment

Because tumors develop ischemic conditions when sorafenib exerts its anti-angiogenic effect, the monitoring of hemodynamics by computed tomography or magnetic resonance imaging may provide useful evaluation criteria on whether to continue drug administration. Our investigation showed that survival was significantly prolonged in HCC patients with necrosis or decreased blood flow, even if only partial, compared with that in HCC patients with no change in blood flow [26].

In conclusion, it is not currently possible to predict treatment outcome or the likelihood of adverse effects of sorafenib treatment before administering the drug. Despite the discovery of genes that are potential biomarkers, such as FGF3/4, these genes have not been put to practical use: current clinical practice consists of starting sorafenib administration and monitoring patients individually to determine the treatment effects. In particular, we need to monitor the radiological response and response of tumor markers (AFP and PIVKA-II) carefully during the initial phase of drug administration (approximately 4 weeks.) At the time of evaluating the treatment effect (after 8 weeks), it may be considered to discontinue administration in patients who have not responded to sorafenib since the likely result will be progressive disease. However, in the real world clinical practice in those patients with slow growing HCCs (long stable diseases), administration of sorafenib even beyond progressive diseases should be continued since there is no second line targeted agents available at the present and maybe sorafenib is beneficial for prolonging survival of such patient population.
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