### SUMMARY

Reliable diagnosis at early stages of disease could improve the chances of survival for patients with lung or liver cancer. Scientists at the Fraunhofer ITEM are making use of genomic and proteomic technologies to identify specific diagnostic biomarkers. Three proteins have so far been identified as specific biomarker candidates for hepatocellular carcinoma, and experiments with transgenic mice revealed some promising proteins with regard to lung cancer, too.

### Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver. Its incidence in Western countries has increased quite dramatically over the past 20 years. Detection of HCC can be difficult, as most of the patients who develop this tumor have no symptoms other than those related to their longstanding liver disease. The onset of abdominal pain, weight loss, early satiety, jaundice, and a palpable mass in the upper abdomen usually indicate an already advanced cancer. Studies performed in several countries have demonstrated that the periodic use of abdominal ultrasound and determination of alpha-fetoprotein (AFP) as a blood tumor marker may enable early detection of small hepatocellular carcinomas in high-risk patients. Unfortunately, serum AFP levels are normal in 40 percent of patients with hepatocellular carcinoma of less than 2 cm in diameter and in 28 percent of those with tumors of 2 to 5 cm in diameter. Moreover, not all hepatocellular carcinomas secrete abnormal AFP. On the other hand, AFP may be elevated also in pregnancy, in the presence of other tumors of gonadal origin, and even in acute or chronic viral hepatitis without a tumor. Since diagnosis at early stages of disease would improve overall survival and imaging and other non-invasive methods are still not sufficiently sensitive, there is a tremendous need to identify specific HCC protein biomarkers.
Proteomics and biomarkers

Substantial progress in functional genomic and proteomic technologies has opened up new perspectives in biomedical research. New classes of diagnostic markers have been developed based on patterns of genomic information. Certain proteomics data are of importance in characterizing the flow of information within the cell and the organism, proteomics being the study of protein expression patterns, protein interactions, and protein pathways in the blood, individual organ systems, and tissue cells. The heterogeneity of both patients and disease states, however, makes it difficult to identify specific biomarkers. The search for regulated serum proteins in a genetic model of liver cancer, therefore, may facilitate the identification of novel biomarkers.

Identification of specific biomarkers

The aim of this project is to identify specific biomarkers for early detection of HCC. A c-Myc-transgenic mouse model is used for this purpose, because Myc overexpression has been observed in the pathogenesis of many cancers including liver cancer, while impairment of Myc hyperactivity was sufficient to stop tumor growth. Mice at different stages of disease are studied to identify proteins regulated at these different stages.

Methods

A well-established proteomic workflow is applied by the ITEM scientists to study differential protein expression in serum from wild-type and c-Myc-transgenic mice (Fig. 1). Two-dimensional electrophoresis is used to separate serum proteins according to their isoelectric points (pI) and masses (Mw). The 2D spots are subsequently analyzed by using an imaging software, digested with the enzyme trypsin, and identified by mass-spectrometric analysis (MALDI-TOF MS). Additionally, Western immunoblotting and immunohistochemistry are carried out to further validate the findings.

Results

Up to now, the study has revealed regulation of 17 mouse proteins in murine HCC; to our knowledge, three of these have not yet been reported in the literature. These proteins – which are biomarker candidates – were also investigated in human serum and liver tissue and were found to be similarly regulated there. Translational research is now on its way to “translate” these results of basic research into the clinic.

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Fig. 1: Analysis of expressed proteins in serum

Histopathology
Control (wild-type mouse)  Tumor (AAT-c-Myc mouse)

2-D electrophoresis
Serum and tissue

Mass spectrometry
Trypsin digestion  MALDI-TOF/TOF MS

Data analysis and data validation
WB: Western blotting  IHC: Immunohistochemistry