

Molecular classification of HCCs: new insights and interest in clinical practice

Most of the HCCs are developed in cirrhotic liver. Cirrhosis of any origin and dysplastic nodules have long been considered to be the likely precursors of HCC because of their frequent association with the HCC occurrence. As in other solid tumors, a large number of genetic alterations accumulate during the carcinogenetic process. Altogether, HCC development resembles to other solid tumors because it is a multi-step process, but it is also a particular process due to the large number of different risk factors that are associated with tumor occurrence. They mainly include infection with the hepatitis B virus (HBV) or hepatitis C virus (HCV), heavy alcohol intake, prolonged dietary exposure to AFB1 or vinyl chloride and primary hemochromatosis. In vast majority of the HCC cases, at least one of these risk factors can be identified, either alone or in combination and the presence of each risk factor among patients varies according to the geographical origin of the patients [6]. This natural clinical diversity of HCCs is also found at the genetic level and as expected, a large number of genetic alterations have been described in HCC, some of them are specific of risk factors. Finally, each HCC tumor shows a broad variety of accumulated genetic and epigenetic alterations.

In fact, as proposed by Vogelstein in overall cancer, HCC is a DNA disease due to the accumulation of genetic and epigenetic alterations and more specifically of genes that control cell cycle and cell proliferation. Some of the observed genetic alterations are widely shared among the different tumor types, however the association of the recurrent genetic alterations is highly specific of a tumor type. Therefore the comprehensive knowledge of a broad field of genetic alterations in a tumor type and the study of the correlation between these alterations and the different clinical and histological parameters allow to refine the tumor classification and the understanding of the multistep carcinogenesis process.

Altogether, genetic alterations accumulated in HCC are numerous with over 20 different genes involved affecting at least 4 principal signaling pathways. To understand the underlying mechanisms between these various genetic alterations, we analyzed a series of 137 French HCC tumors related to various risk factors. We found that chromosome instability together with *TP53* and *AXIN1* mutations were closely related to HBV infection. In contrast the second hepatocarcinogenesis pathway defined by β -catenin mutation associated with chromosome 8p deletion in a context of chromosome stability is significantly associated with the absence of HBV infection. Furthermore, chromosome instability measured by the fractionnal allelic loss (FAL) was an independent prognostic marker in resected HCC.

In a more recent study, we performed a genome wide transcriptomic analysis of 60 HCC tumors together with an exhaustive characterization of structural genetic alterations and clinical parameters. In this study, unsupervised transcriptomic analysis identified six robust subgroups of HCC (termed G1 to G6) associated with clinical and genetic characteristics. The main classification divider was chromosome stability status. Tumors from group G1 to G3 were chromosome instable whereas tumors from G4 to G5 were chromosome stable (G4 to G5). Indeed, tumors presenting chromosome instable phenotype demonstrated a transcriptomic profile strikingly different from chromosome stable ones. Once again chromosome instability appears as the main driver of tumor classification. In addition, genetic alterations and

pathways analyses allowed for a refined transcriptomic classification: G1-tumors were related to a low copy number of HBV and overexpression of genes expressed in fetal liver and controlled by parental imprinting; G2 included HCC infected with a high copy number of HBV, *PIK3CA* and *TP53* mutated cases; G3-tumors were *TP53* mutated without HBV infection, a frequent P16 methylation and showed over-expression of genes controlling cell-cycle; G4 was a heterogeneous subgroup of tumors including *TCF1* mutated adenomas and carcinomas; G5 and G6, were strongly related to β -catenin mutations leading to Wnt pathway activation; G6-tumors presented satellite nodules, higher activation of the Wnt pathway and a E-cadherin under-expression. This 6-group classification has clinical application regarding the development of targeted therapies for HCC because specific pathway activations, particularly AKT and Wnt pathways, are closely associated to subgroups G1-G2 and G5-G6 respectively. Therefore we identified and validated a robust 16-gene signature to classify HCC tumors into the 6-group transcriptomic classification. This signature should be very useful to determine alterations of specific pathways and to predict putative response to targeted drugs.

Conclusion: Hepatocellular carcinoma has been extensively studied in term of genetic alteration in the last 10 years and our knowledge has dramatically increased in this field leading to the definition of the different altered pathways in hepatocarcinogenesis. Transcriptomic classification is closely related to tumor genotype. In fact, tumor suppressor gene inactivation and activation of oncogene, by somatic gene mutation or epigenetic modifications are strongly linked to the different pathway alterations. Global classification using molecular signature will be very important to define homogeneous sub-group of tumors with similar genotype and combination of altered pathways. Moreover, in the future, the best use of targeted drugs will require extensive definitions of the genotypes of the tumors. In this context, molecular classification, particularly using transcriptomic signature are promising tools to understand and then to predict efficiency of those novel drugs, leading to a “à la carte” treatment.

Major references:

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