Strategies for HCC therapy and diagnostics – lessons learned from high throughput and profiling approaches

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Abstract

Over the last decade numerous small and high dimensional profiling analyses have been carried out in human hepatocellular carcinoma (HCC) addressing different levels of regulation and modulation. Since comprehensive analyses are lacking, the following review tries to summarise some of the general results and to compare it with insights from other tumor entities. Specific attention will be given to their impact on future diagnostic and therapeutic approaches.
Introduction

Hepatocellular carcinoma (HCC) is a unique tumor entity by several means: Its causes (chronic viral hepatitis, alcoholic and non-alcoholic steatohepatitis, aflatoxins, several hereditary diseases, e.g. genetic haemochromatosis) are much better defined than in other adulthood cancers and are demonstrable in about 90% of the cases. Consequently, HCC is the most relevant paradigm of virus- and inflammation-associated cancer. This opens a large field for primary (immunisation, specific hygienic measures) and secondary prevention (screening programs, therapy of predisposing diseases) but it is also the reason of another peculiarity: conflicting morbidity. The majority of HCC patients, in highly industrialized countries over 80%, have chronic hepatitis or cirrhosis, which influences disease progression and may severely restrict patient prognosis, therapeutic options, and design of clinical trials.¹ The morphological sequence of premalignant and early malignant changes is well defined but the lesions are hardly accessible by diagnostic means,² which constitutes a significant difference to colon, breast, or skin cancer. Well characterized model systems are available for mechanistic as well as preclinical analyses³ and HCC cell lines have been workhorses for biochemical as well as molecular biological, and recently even systems biological analyses, providing a wealth of basic research data for current translational approaches.

Due to the obstacles characterized above, HCC has long been an orphan tumor disease in regard to translational research efforts, clinical trial perspectives, and therapeutic options, which stood in sharp contrast to its enormous clinical relevance. HCC is the sixth most frequent cancer and the third most frequent cause of cancer-related death and numbers are rising even in the industrialized countries.⁴ Nevertheless, knowledge about molecular pathogenesis of HCC is lagging behind other major tumor diseases, such as breast and colon cancer, where multiple systemic treatment options are starting to convert in many cases previously untreatable metastatic tumors into a chronic disease. Recently, for HCC
this picture has started to change and has taken some momentum: The growing Chinese economy has fuelled industry’s interest and improved options for clinical trials and novel therapeutics. The successful SHARP trial has established Sorafenib as the first effective and approved systemic treatment against HCC and has proven that despite all obstacles, trials employing systemic treatments can be successful.\textsuperscript{5} Other treatment options, such as radioembolization\textsuperscript{6} and oncolytic approaches\textsuperscript{7} have entered the field. Meanwhile, over 150 phase I-III trials are ongoing (source: http://clinicaltrials.gov), but only some of them are based on rational approaches using the knowledge about molecular pathogenesis of human HCC. Comprehensive, large scale profiling approaches on representative collectives are missing so far, but numerous analyses have been carried out at the genomic, epigenetic, and expression level providing first insight into relevant mechanisms, targets, as well as markers suggesting some future strategies for systemic HCC treatment.

Technical aspects

Genomic, miRNA, and some protein based assays are more robust and less vulnerable to influences imposed by imperfect sample conditions. These approaches are well applicable to formalin-fixed paraffin-embedded (FFPE) samples and thus have found their way even into routine diagnostics. In contrast, assays based on larger coding or non-coding transcripts highly depend on material preservation and assay conditions. This does not restrict their potential as exploratory technologies but impedes their comparability and severely restricts meta-analyses and diagnostic applicability. Currently, methylation analyses pose significant challenges in data acquisition as well as interpretation. Broad spectrum proteomic or metabolomic approaches are certainly further away from application and have not been used for significant HCC collectives.

Basically, profiling data analyses can be carried out in unsupervised and supervised fashion. Although unsupervised analyses are believed to be less biased, in most cases
geographic parameters or the fact that e.g. only resection specimen are used inherently influences data interpretation. But due to e.g. profound knowledge about its etiology, translational HCC research needs hypothesis-driven, supervised analyses guided by epidemiological, clinical, or experimental nominators to identify factors modulating its development or progression. These factors may include viral and non-viral etiology,\(^8\) gender,\(^9\) tumor recurrence,\(^10\) intrahepatic metastasation,\(^11\) response to therapy,\(^12\) and fetal type gene expression pattern.\(^13\) Knowing and controlling this bias impeding all supervised and unsupervised HCC analyses is of utmost relevance for drawing conclusions and making strategic decisions.

Source of the tissue samples is an important bias since etiology varies dramatically depending on the geographic region of origin.\(^4\) HBV etiology is less frequent and aflatoxin-based effects are usually absent in collectives from Western industrialized countries compared to Far Eastern Asian and Southern African while effects of alcohol consume and metabolic syndrome are more prevalent. Furthermore, significant differences exist in the relative frequency of HBV- vs. HCV infection. In addition to geographic differences, collectives based on resection specimen in general address limited disease and are biased for non-metastatic, less aggressive tumors of presumably better spontaneous course, and also for a lower frequency of cirrhotic changes in the non-tumorous liver. These factors have already been demonstrated to correlate with differences in the results of the respective analyses; thus, we currently have no single analysis in hands which is really unbiased. Consequently, many array-based analyses have obtained inconsistent and partly contradictory results. One possibility to control this problem are meta-analyses integrating as many data from different studies as possible or reflections comparing results from different types of studies. Unfortunately, these approaches suffer from variations in regard to technical platforms, sample preparation and evaluation, normalization, as well as data analyses; they have successfully been performed with robust genomic data but
recently some success has also been achieved using transcriptomic HCC data. There is a strong need for comprehensive analyses, addressing several levels of regulatory processes in a single collective and for analyses of collectives that are less biased; the results are likely to differ from those obtained so far. Whether ongoing large scale but still biased efforts for systematic analysis of cancer genomes, such as ICGC (International Cancer Genome Consortium) will improve this specific situation in HCC has to be seen.

Screening for Aberrations

Genetic Analyses

Historically, comparative genomic hybridization (CGH) represented the first molecular method to screen tumor tissue for genetic changes in a comprehensive manner. More than 40 single studies in human HCC have elaborated recurrent chromosomal imbalances which correlated with etiology (e.g., losses of 4q, 8q, 13q, and 16q with HBV; losses of 8p in HCV-negative cases) or tumor progression (losses of 4q and 13q). Self-organizing tree algorithms identified gains of 1q21-23 and 8q22-24 as early and the gain of 3q22-24 as late genomic events, demonstrating sequential gain of genetic instability. In contrast to conventional CGH, array-CGH (aCGH) approaches provide higher genomic resolution and therefore allow to scale down the correlations of more and smaller aberrations with clinicopathological features such as microvascular invasion and tumor grading. Moreover, specific alterations (e.g., 1q32.1, 4q21.2-32.33) discriminate between HBV- and HCV-associated HCCs, and certainly the high resolution of this technique allows for the precise delineation of respective candidate oncogenes and tumor-suppressor genes as demonstrated e.g., for Jab1, YAP, and Mdm4. In summary, three main conclusions can be drawn from these studies: i, HCC is a chromosomal instable cancer in general accumulating high numbers of macro- and micro-imbalances. ii, early chromosomal imbalances precede malignant transformation as they are detectable in a significant
number of premalignant lesions. iii, etiology matters, as several chromosomal macroimbalances correlate with the underlying cause of the HCC. The reason for this observation has not been clearly defined.

Mutational activation and inactivation of individual genes are frequently observed in most HCCs and represent protumorigenic events independent of genomic instability. Here, especially loss of function as well as gain of function mutations in TP53 facilitate tumor cell mitosis and cell survival. In addition, several mutations with low or moderate frequency have been described for HCC e.g., in AXIN1/2, CTNNB1, M6P/IGF-2R, TCF1/HNF1α, PIK3CA, K-RAS, and p16/CDKN2/INK4A (Table 1). Data collected so far demonstrate that few high frequency mutations and many low frequency events contribute to the molecular heterogeneity of HCC. The spectrum of mutations will certainly be expanded by high-throughput sequencing technology in the near future.

**Epigenetic Analyses**

In carcinogenesis, global DNA hypomethylation has been associated with activation of oncogenes and genomic instability, while hypermethylation of CpG islands located especially in gene regulatory sequences (e.g., of the Ras target RASSF1A, the adhesion molecule CDH1, and the cell cycle regulator p16/CDKN2/INK4A) resulted in transcriptional silencing. Methylation changes may occur early in the process of cancer development and CpG island hypermethylation of regulatory regions of tumor-relevant genes is a frequent event accumulating in multistep hepatocarcinogenesis. Only few studies have analyzed the global and promoter-specific levels of DNA methylation in hepatocarcinogenesis. First published data revealed clear differences in DNA methylation between HCC and surrounding non-tumorous tissue based on specific promoter hypermethylation and global hypomethylation. In this regard, genomic hypomethylation correlated with genomic instability in HCC while CpG promoter methylation was associated
with poor prognosis.\textsuperscript{33} In addition, DNA methylation status correlated with tumor recurrence after hepatectomy, cancer-free, and overall survival.\textsuperscript{34} Using class comparison analysis, HBV-, HCV-, and alcohol-specific promoter methylation pattern have been described suggesting etiology-dependent methylation in early stages of hepatocarcinogenesis.\textsuperscript{32} Based on the premature appearance of these modifications in tumorigenesis, epigenetic analyses may represent a valuable tool for diagnosis and classification in early stages of liver tumor development.

**Transcriptional Analyses**

*Coding Transcripts*

Most transcriptomic studies in HCC have used cDNA or oligonucleotide high-density microarrays. Despite of varying technical platforms, biological controls, and mathematical algorithms, these approaches have identified partly novel tumor-relevant genes and networks (e.g., PEG10, IGF-II, Claudin10, RhoC, AP-1, and cell cycle regulators).\textsuperscript{14,35-37} Some studies have correlated expression profiling data in HCC with etiology,\textsuperscript{8} vascular invasion,\textsuperscript{38} drug response,\textsuperscript{13} recurrence,\textsuperscript{12} and survival.\textsuperscript{36} Unsupervised clustering of transcriptomic data provided subtyping of HCC that was related to tumor-associated inflammation as well as tumor cell proliferation and apoptosis.\textsuperscript{35,39} Furthermore, specific expression signatures derived from global gene expression analyses correlated well with the histological classification of premalignant lesions (low and high grade DNs) and HCCs.\textsuperscript{40} Ye et al., also demonstrated that transcriptomic signatures significantly differed between HCCs with and without metastatic spread while expression profiles of respective primary and metastatic tumors varied only by few genes.\textsuperscript{41}

Hierarchical clustering has revealed that HCCs can be divided into subgroups based on transcript profiles. Lee et al., described the existence of two distinct groups of HCC
characterized by poor survival specifically within the group that showed high expression of genes involved in proliferation and anti-apoptosis.\textsuperscript{36} This study further demonstrated that the transcriptional pattern of HCCs that shared a signature with fetal hepatoblasts exhibited poor prognosis.\textsuperscript{14} Yu et al., demonstrated an association between their identified subclasses and tumor dedifferentiation (grading G1/2 vs. G3/4) as well as overall survival.\textsuperscript{42}

Despite all limitations a recent meta-analysis integrating a high number of HCC data (>600) from independent gene expression profiling analyses was able to demonstrate the existence of 3 distinct molecular subclasses (S1-S3) and confirmed some important previous findings (e.g., activation of Wingless-pathway, existence of a proliferation signature). However, it also exemplified some of the difficulties ahead by e.g. showing that activation of typical Wingless-dependent gene expression did not correlate with mutations in \textit{CTNNB1}.\textsuperscript{16} In summary, transcriptional signatures have allowed for classification of HCCs according to their molecular and biological characteristics\textsuperscript{26} and have turned out to represent a valuable tool to identify tumor-relevant genes and pathways in human HCC.

\textit{Noncoding RNA/microRNA}

A steadily increasing number of studies has examined differential expression of non-coding RNAs (especially microRNA, miRNAs) in HCCs. miRNAs bind complementary sequences in the 3'-end of mRNAs and therefore represent effective posttranscriptional regulators of mammalian gene bioactivity.\textsuperscript{43} In addition, miRNAs directly affect promoter activity through binding and or modifying DNA methylation.\textsuperscript{44-45} So far, miRNAs with oncogenic or tumor-suppressing potential have been identified,\textsuperscript{46-47} and recent results indicate that different stages of hepatocarcinogenesis as well as liver tissues with HBV- or HCV-infection can be differentiated from each other based on their miRNA fingerprints.\textsuperscript{48} This is supported by other studies showing that distinct miRNA signatures were associated
with alcohol consumption and HBV-infection, tumor differentiation and progression, metastasis, survival, and relapse. Although identified key miRNAs significantly differed between various studies, some miRNAs such as miR-122 and miR-223 have recurrently been identified by independent approaches. Using specific antagonirs and agomirs it is possible to associate distinct miRNA activity with cellular processes, but since each miRNA potentially interacts with several different targets, it is difficult to define the precise mechanism and pathways by which miRNAs mediate their biology. However, recently reduced miR-26 expression was linked to NF-kB and IL6 signalling, shorter survival, and better response to IFNα therapy. In addition, independent studies demonstrated the relevance of other miRNAs such as miR-139, miR-125b, miR-221, and miR-181 in the regulation of tumor-relevant proteins and processes in hepatocarcinogenesis. Recent data demonstrated, that on the basis of c-MYC-dependent miRNA signatures detected in hepatoblastoma, it is possible to discriminate between HCCs with an invasive phenotype and patients with lower survival probability. Based on their high stability even in FFPE tissues miRNAs represent promising molecular markers for diagnostic HCC classification, prognostic stratification, and drug-response prediction even under routine clinical conditions.

Protein Analyses

Proteomic analysis (e.g., by 2D gel electrophoresis, MALDI- or SELDI-TOF) is believed to be more informative than other screening approaches since proteins represent the main functionally active principle in cells. Moreover, only moderate correlations between mRNA and protein abundance demonstrate the value of protein assays for biomarker identification in blood and liver tissues. For HCC, distinct protein profiles discriminating between HBV- and HCV-associated tumors have been identified. In addition, many differentially expressed proteins (partly derived from patients sera) in HCCs have been
described including e.g. HSP90\textsuperscript{63} and stathmin.\textsuperscript{64} However, since in the different studies the number of identified proteins is relatively low (normally far below 100), protein-based assays have been of limited value for subtyping of HCCs so far.

Many studies have used immunohistochemistry for the analyses of distinct factors and protein families in HCC. These include signalling pathway constituents (e.g., β-catenin,\textsuperscript{24} different FZD receptors,\textsuperscript{65} and c-MET\textsuperscript{66}), cell cycle regulators (e.g., p53,\textsuperscript{24} p16/CDKN2/INK4A,\textsuperscript{27} and survivin\textsuperscript{67}), as well as transcriptional regulators (e.g., c-MYC,\textsuperscript{24,68} FBPs,\textsuperscript{69} YAP,\textsuperscript{70} and SMADs\textsuperscript{71-72}). In addition, these analyses discriminate between different subcellular localizations of proteins, e.g. for β-catenin or p53, and provide the possibility to assess protein expression in semiquantitative manner using high throughput imaging systems and respective mathematical analysis algorithms. In conclusion, protein-based assays may provide the most relevant information with regard to levels and location of bioactive units in cells; however, technical limitations currently prevent these approaches from being available for unbiased analyses in larger HCC collectives.

**Integrative Approaches**

Integrating different types of analyses in HCC has supported the identification of genes and pathways that are often aberrantly regulated by several different low frequency mechanisms. For example, aberrant activation of pleiotropic growth factors, receptors, and their downstream signaling pathway components represents a central protumorigenic principle in hepatocarcinogenesis. Constitutive dysregulation of these pathways (e.g., HGF/c-MET-, IGF/IGF1R-, TGFα/EGFR-, TGFβ/TGFR-signaling) occurs at different mechanistic levels, such as regulation of expression, cell-type specific and subcellular localization, and mutational (in)activation.\textsuperscript{73-74} In addition, cross-talks between pathways and with other tumor-relevant factors (e.g., HSPs, COX-2, p53) further demonstrate that
integrative approaches including genomic, transcriptomic, and protein analyses are necessary to understand the complex and dynamic interplay between different oncogenic modules and of pro- and anti-oncogenic mechanisms.

Moreover, integrative approaches have started to support classifying groups of HCCs according to specific overall molecular characteristics, prognostic impact, and even some predictive implications. Laurent-Puig et al., identified two molecular subgroups characterized by high chromosomal instability (associated with *Axin-1* and *TP53* mutations) or more stable conditions (associated with *CTNNB1* mutations). HCCs from the first group were less differentiated, more frequently exhibited HBV-infection, and in case of LOH of 9p and 6q showed poorer prognosis. Based on aCGH analyses, Katoh et al., equally identified genetically homogenous classes of HCC (2 clusters and 6 subclusters). In contrast to the previous study, specific chromosomal alterations (gains of 1q, 6p, 8q and losses of 8p) in one cluster were associated with high chromosomal instability and poor patient survival. In addition, no correlation of *CTNNB1* or *TP53* mutations to any of the groups was detectable. Of relevance, some subclusters harbored genomic amplifications of genes involved in mTOR- and VEGF-signalling. Based on the integration of genomic data of copy numbers and gene expression profiles, Woo et al., identified potential 50 driver genes in HCC. In fact, tumor classes defined by the expression of this signature predicts the prognostic outcome of HCC patients.

Boyault et al., described the existence of 6 HCC groups (G1-G6) that were characterized by distinct clinical and molecular features. For example, G1-G3 tumors exhibited more chromosomal instability and a tendency for poorer prognosis than G4-G6 HCCs. *TP53* mutations accumulate in the subgroups G2/G3 while mutations in *CTNNB1* are characteristic for G5/G6 tumors. Interestingly, this grouping showed some similarities with the molecular classification of Lee et al., like the existence of groups with chromosomal instability, poor survival, and hepatoblast characteristics. By integration of genomic,
transcriptomic, and protein information of HCV-associated HCCs, Chiang et al., defined five molecular classes of HCC that partly overlapped with previously described groups. These classes were characterized by known molecular features such as activation of the Wingless-pathway, proliferation, chromosomal instability, and induction of IFN-stimulated genes. In addition, this study identified a new subgroup characterized by polysomy of chromosome 7. Overall there is increasing evidence that robust and clinically relevant subclassification requires integration of different technologies; future analyses will have to elaborate minimal diagnostic pattern in order to allow rational upfront testing.

Lessons learnt from profiling analyses

Although comprehensive analyses of all aspects in a representative collective of HCCs are missing and the existing data are either incomplete or biased, several conclusions can be safely drawn at this time point:

1. The vast majority of HCCs represents a classical chromosomal instable tumor (CIN-phenotype), carrying multiple genomic imbalances, comparable to other adulthood malignancies, such as breast and sporadic colon carcinoma, or pancreatic cancer. Microsatellite instability (mismatch repair deficiency) and methylator phenotypes seem to have little if any overall impact. Chromosomal instability increases during tumor progression suggesting it as a driving factor. Since genomic macro-imbalances at some genomic loci (but not global chromosomal instability) can be observed already in premalignant Dysplastic Nodules, an important question is, which processes force chromosomal instability or govern relative chromosomal stability and at which stages during tumor progression they become altered. There is experimental evidence for p53-dependent senescence surveillance to contribute to chromosomal stability, but it is unlikely to be the only factor. Molecular mechanisms of chromosomal stability governance
may not only offer insights into mechanisms of HCC progression but may also be valuable therapeutic targets even in progressed, chromosomal instable HCC.

2. Somatic mutations in HCCs (in addition to chromosomal imbalances) include point mutations, small/medium size insertions and deletions, as well as balanced chromosomal translocations. Some of the HCC mutations are of high frequency (such as codon 249 mutations of p53 gene in aflatoxin exposed collectives), but the vast majority of these mutations affect only small subpopulations of HCCs, as for example demonstrated for Axin-1 mutations. Mutational analyses of single genes in different HCC populations underscore the molecular heterogeneity of human HCCs as demonstrated also by chromosomal imbalances. Comprehensive mutational data for HCC collectives are lacking so far and will be collected in the frame of the ICGC. First deep sequencing data have been obtained in other adulthood type cancers, such as colon, breast, and pancreatic cancer, where mutations in coding regions of cancer genomes average numbers of about 60-80 mutations and in some cases may even reach triple digit numbers. Thus the number of mutations is much higher than previously expected; this is even more evident in the light of the fact that these mutations are complemented by epigenetic changes and gross chromosomal imbalances. The situation becomes more complex considering the impact of these mutations, since data from breast and colorectal cancer suggest that some of them are driver mutations, while the vast majority of mutations may be associated only with small fitness advantages. There is little doubt that the situation in HCC will be comparable, but the specific impact for tumor therapy is unknown and remains to be analyzed.

3. There is convincing evidence, that etiology leaves its molecular traces in the tumor genome leading to specific genomic imbalances, mutations, epigenetic changes and resulting alterations in host gene expression. Whether these effects are direct consequences of the specific carcinogenic mechanisms (e.g. exerted by HBV-integrations...
or direct genotoxic effects of mycotoxins) or represent indirect effects due to functional selection of complementary protumorigenic mechanisms cannot be answered globally. Nevertheless, etiological 'fingerprints' offer insights into the stepwise process of molecular carcinogenesis, the interrelation of different oncogenic mechanisms, and provide openings for secondary preventive strategies. HCC was one of the first and is certainly one of the best studied paradigms for molecular cancer epidemiology\textsuperscript{81} and may thus fuel the search for further, so far undetected etiological mechanisms.

4. Comprehensive approaches in other tumor entities, such as breast, colonic and pancreatic cancers have convincingly shown that in common solid cancers of adulthood a surprisingly high number of pathways (around 12-15) is altered in a protumorigenic manner.\textsuperscript{83-84} These different, affected pathways seem to cover most of the tumor relevant functions, but at the same time significant functional overlaps exist between them.\textsuperscript{74} As outlined above, current evidence for HCC points in the same direction. Frequently affected pathways and effectors include Wnt-signalling, growth factor-induced signalling (e.g., IGF and TGF\textbeta), or cellular gatekeeper such as p53. Matching affected pathways and underlying molecular changes shows that these pathways can be altered at different points, which has already been proven e.g. for Wnt/wingless signalling (e.g., Axin-1/2 and \textbeta-catenin mutations, increased cadherin-17).\textsuperscript{23-24,82,85} Interestingly, growth factor research in HCCs has shown that in each given pathway frequent, typical alterations (nodal points?) exist, but these changes differ between the pathways, varying from aberrant ligand expression (e.g. IGF-II)\textsuperscript{86} over receptor bioavailability (e.g. c-MET)\textsuperscript{66} to alterations in intracellular signal transducers (e.g. TGF\textbeta-signalling).\textsuperscript{71-72} The cause for these observations is unknown, but it may offer some hints, how therapeutic approaches should be designed in order to target essential points of interference. Finally, it is reasonable to conclude, that the spectrum of molecular changes present in a given HCC also contains stochastic elements largely selected by function. There is no evidence for a dominant
driver mechanism and resulting addiction to it as can be observed e.g. in several childhood malignancies and gastrointestinal stromal tumor.

5. Comprehensive analyses have started and are likely to provide molecular subgrouping of HCC. Initial attempts have been made e.g., by J. Zucman-Rossi and her group, clearly demonstrating the feasibility of the approach. Improvement can be expected from further meta-analyses of existing data and novel comprehensive analyses on well characterized collectives. There is significant evidence that molecular classification reflects functional aspects and correlates with prognosis. At least some of the subgroups are likely to be relevant for therapy and predictive diagnostics, as exemplified by IGF-IR and mTOR-associated signalling.

**Consequences for therapeutic approaches**

What are the consequences for drug development, clinical trials, and molecular (predictive) diagnostics? There is certainly sufficient room and need for further (pathway) targeted approaches. Constitutive activation e.g., by mutation or ligand based stimulation of growth factor signalling pathways is a common theme most likely relevant in every single HCC. On the other side many different pathways can be affected and their functional consequences in regard to proliferation, motility, anti-apoptosis, and angiogenesis, are significantly overlapping. Thus, response to specific tyrosine kinase-directed approaches may be limited, can be expected only in subgroups of HCCs, and secondary resistance is likely to occur soon, since there is little if any evidence for a specific pathway addiction in HCC. From a mechanistic point of view approaches to inhibit tyrosine kinases/growth factor signalling pathways should be as broad as possible and should upfront consider complementary and combinatorial settings. Identification of patients that may benefit (more) from these approaches requires comprehensive biomarker analyses accompanying
the clinical trials. This is state-of-the-art in most other malignancies, but has not been thoroughly respected in HCC, probably due to the fact that HCC is the only relevant tumor entity that does not necessarily require tissue based diagnosis prior to therapy. Since molecular definition of responsive subgroups is not possible without tissue access, this difference may cause more trial failures than expected and necessary and may turn out to be a negative aspect of HCC in comparison to other tumor entities.

The fact that protumorigenic alterations in relevant pathways in HCCs may occur at different (nodal) points may limit the application of specific inhibitors and has to be respected in predictive diagnostic approaches as well as drug and subsequent trial design. A question that has to be always addressed is the size of the responsive patient collective and whether it justifies the clinical and commercial effort. Some of these aspects can be better addressed by novel adaptive trial concepts. It also has to be considered that drug companies may rather accept to lower the therapeutic benefit than the size of the patient collective amenable to treatment.

Since breakthrough achievements are unlikely to result from specific (pathway) targeted approaches in HCCs, our attention should focus also on mechanisms that are constantly needed by the tumor (‘Achilles heels’). These represent either necessarily required cellular functions that support a protumorigenic phenotype or are central mechanisms that allow for tumor persistence or progression. Examples for the first are the chaperone network (e.g. HSP90 and interacting factors) as well as all factors that support tumor cell proliferation and cell cycle progression. Tumor associated neoangiogenesis may represent a double edged sword; on one hand it is an indispensable prerequisite for tumor growth, on the other hand it is required to build up sufficient intratumoral drug concentrations. Recent results indicate that the effect of antiangiogenic approaches may depend on tumor characteristics (e.g. tumor cell biology and stroma content) that may need further attention. Examples for central dysregulatory or tumor tolerance mechanisms may
provide an even more attractive basis for therapeutic concepts. Global downregulation of miRNAs is found in most tumors and suggest a role for the miRNA processing machinery. There is recent evidence for a critical role of dicer and some link to the p53 family members. It will have to be shown, whether this holds true in HCC and can be modulated in an anti-neoplastic manner. Tumor cell aneuploidy, as present in almost all HCCs, is a condition usually not compatible with cell survival under physiological conditions; this may explain the usually higher apoptosis rate of malignant tumors, but tumor cells must have also established mechanisms to prevail and maintain all vital cell functions despite the presence of significant aneuploidy. First screens have demonstrated genes that may provide increased aneuploidy tolerance; the future will show whether they may represent valid and innovative drug targets.

These considerations provide different challenges for drug design. Tumor cell specificity may not be achieved by addressing pathways or specific mechanisms that are more or less exclusive to tumor cell; instead pharmacokinetics and pharmacodynamics may have to be modulated in order to favour tumor cell-associated activity or activation of the drug employing tumor preferential mechanisms.

Predictive marker analyses do not play a role in current clinical diagnostics in HCC, but it will be necessary to include them in future clinical trials. Even if broader therapeutic approaches are tested, predictive marker analyses may well indicate response as well as primary and secondary resistance to therapy. Paradigms are e.g. MSI-testing for chemotherapy response in colorectal cancer and ERCC1-testing regarding platinum-based chemotherapy in NSCLC. Clearly the value of histological subtyping and molecular predictive diagnostics exceeds target gene evaluation.

Knowledge about molecular pathogenesis of HCC has dramatically improved over the last years and some progress has been made (or is just ahead) in translation into clinical application, but there is room for improvement. Especially comprehensive molecular
analyses and further rationally designed clinical trials based on molecular evidence (e.g., targeting IGF-IR and mTOR) are eagerly awaited.
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*vinyl chloride exposition*
References


