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Correlation of molecular alterations with pathological features in hepatocellular carcinoma: literature review and experience of an Italian center

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Abstract

Hepatocellular carcinoma (HCC) represents the primary carcinoma of the liver and the fourth leading cause of cancer death. The World Health Organization estimates an increase in cases in the coming years. The risk factors of HCC are multiple and the incidence in different countries is closely related to the different risk factors to which the population is exposed. The molecular mechanisms that drive HCC tumorigenesis are extremely complex, but understanding this multi-step process, is essential for the identification of diagnostic, prognostic, and therapeutic markers. The development of multigenic NGS panels through the parallel analysis of multiple markers can provide a landscape of the genomic status of the tumor. Considering the literature and our preliminary data based on 36 HCCs, the most frequently altered genes in HCCs are *TERT*, *CTNNB1*, and *TP53*. Over the years, many groups have attempted to classify HCCs on a molecular basis, but a univocal classification has never been achieved. Nevertheless, statistically significant correlations have been found in HCCs between molecular signature and morphologic features and this leads us to think that it would be desirable to integrate the approach between anatomic pathology and molecular laboratories.

Key Words: Hepatocarcinoma; Mutation; Next-generation sequencing; Review; TP53; CTNNB1; TERT

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Core Tip: The molecular mechanisms that drive HCC tumorigenesis are extremely complex and a univocal classification, based on molecular features, has not been defined. In the age of precision medicine, the study of HCC mutations is still a field that worth to be investigated. Based on this, we wanted to analyze the possible correlations between molecular alterations and pathological features. Considering both the literature data and our personal experience, about 80% of HCC harbor mutations in at least one among TERT, TP53, or CTNNB1 genes, with different biological and clinical implications

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the world-leading cancers, representing approximately 80% of the primary carcinomas of the liver[1] and the fourth most common cause of cancer-related death. The World Health Organization (WHO) estimated more than 905.500 new HCC cases in 2020 worldwide[2], and based on its projection an increase of 58% is evaluated by 2040 with a total of 1.400.000 new cases and 1.000.000 deaths within 2030[2].

The etiological factors for the HCC development are several: i) infections, including Hepatitis B virus (HBV) and Hepatitis C virus (HCV), with or without co-infection of Hepatitis Delta virus (HDV); ii) lifestyles risk factors and behaviors, such as alcohol addiction and smoking; iii) environment, such as dietary toxins (e.g., aflatoxins, or aristolochic acid); iv) underlying diseases, such as obesity, type 2 diabetes, non-alcoholic liver steatohepatitis (NASH)/non-alcoholic fatty liver disease (NAFLD); v) genetics: some single nucleotide polymorphisms (SNPs) are identified to be associated with HCC risk at different stages, from predisposition to risk factors, to the severity of the chronic liver disease and its evolution to cirrhosis, or to the malignant transformation and tumor progression[3, 4]. For example, a SNP correlated with higher infection risk (MDM2 Promoter SNP309, MDM2 G-309T, rs2279744) has been associated with HCC patients with chronic hepatitis C[5].

The incidence of HCC in different countries varies considering the different risk factors mentioned above. In Eastern Asian countries and most African countries, the incidence of HCC is mostly due to aflatoxins exposure and HBV infection, except for Northern Africa where HCV infection is prevalent[6, 7]. In traditional Chinese herbal medicines, practiced particularly in China, Vietnam, and Southeast Asia, plants containing aristolochic acid are commonly used. In this area, Next-Generation Sequencing (NGS) studies underlined that a fraction of HCCs harbored high rates of mutations matching a distinctive mutational signature of aristolochic acid exposure[8-10]. Moving to Western countries, the incidence of HCC is usually more associated with HCV infection, dietary habits, and related metabolic diseases, such as NASH and NAFLD. In this area, the low

incidence of HCC due to HBV/HCV infections can be explained considering the use of the vaccine for HBV and anti-viral treatments against HCV, in contrast with the increased incidence of metabolic syndrome[11].

All the above-mentioned risk factors lead to liver disease (cirrhosis or chronic inflammation) that causes an accumulation of genomic alterations driving to HCC. In general, HCC arises during the progression of pre-existing chronic hepatitis, and in the vast majority (80%) of patients, the HCC occurs in the setting of cirrhosis[12]. The development of HCC is a process characterized by a specific sequence of lesions, from regenerative nodules in cirrhosis, low-grade dysplastic nodules (LGDN), high-grade dysplastic nodules (HGDN), to early and progressed HCC[13, 14]. The molecular mechanisms driving HCC tumorigenesis are extremely complex. Understanding this multi-step process, with underlying genetic alteration, is essential for prevention, diagnostic, prognostic, and therapeutic purposes. Considering the future perspective, a better knowledge of molecular mechanisms involved in HCC tumorigenesis would help for a correct classification of HCC, for improving patient outcomes, and to develop new therapeutic targets. The advent of NGS technologies may help in the comprehensive study of genetic alteration and the different pathways involved in the initiation and progression of HCC. In fact, the development of NGS multi-gene panels allows the parallel analysis of multiple markers giving a broad view of the genomic situation[15, 16]. To date, this molecular landscape is crucial for therapeutic decision-making in other solid tumors[15]. The Cancer Genome Atlas (TCGA) Research Network investigated a total of 559 cases of HCC[17]. This study found that *TERT, TP53*, and *CTNNB1*, are the most frequently altered genes in HCCs, 77% of HCCs showed a mutation in at least one of these three genes[17]. Correlation data between HCCs molecular signatures and etiological agents are showed in Table 1.

Bearing in mind all this evidence, the present review will discuss the main molecular mutations in HCC, with particular emphasis on the influence that these alterations have on HCC morphology and biological aggressiveness.

MOLECULAR ALTERATIONS IN HCC

TERT

During cycles of genomic replication, the linear organization of chromosomes brings with it the problem of erosion of the 5'-terminus due to non-reproduction of the RNA primer binding site. Indeed, this erosion does not happen thanks to telomerase, constituted by telomerase reverse transcriptase (encoded by the *TERT* gene) and RNA template (encoded by the *TERC* gene). The telomerase complex adds nucleotides onto telomeres, preventing them from shortening. Telomeres are short tandem repeats of DNA (TTAGGG), coated by a protein complex known as Shelterin to protect the end of the chromosome, where telomeres are located. Telomere synthesis is a controlled process activated in stem cells, but deactivated in most somatic cells, due to epigenetics silencing, during the differentiation process. In the mature hepatocytes, the telomerase is not expressed[18, 19]. The shortening of the telomeres exposes chromosomes to damage resulting in cellular senescence and is considered responsible for a sequence of events that drive to cancer[20]. Re-activation of *TERT* expression has been observed in several cancers (e.g., melanomas, gliomas, poorly differentiated bladder cancer, anaplastic thyroid carcinomas, basal cell, squamous cell carcinomas) leading to a restoration of the telomerase activity [19] (**Figure 1**). This event avoids cellular senescence and leads cancer cells to acquire replicative immortality, a crucial feature in the progression of the neoplasm, rather than in the transformation of the cells into malignant ones[21-24]. This up-regulation of *TERT* in cancer can occur through several mechanisms, which are generally mutually exclusive:

gene amplification, found in ovarian cancer, adrenocortical carcinoma, lung adenocarcinoma, oesophageal carcinoma[25];

gene rearrangements, found in high-risk neuroblastoma[26];

gene mutations in hot-spot regions of the promoter region, found in melanomas, thyroid tumors, gliomas [19].

Alterations in the gene promoter region are the most common and most frequently detected, in particular, C>T transition at chr5:1295228 (−124 or C228T) or chr5:1295250

(−146 or C250T). The C228T and C250T TERT mutations create, separately, an identical 11-base sequence that acts as a novel E-twenty-sis (ETS) transcription factor binding site, causing *TERT* over-expression[21].

TERT and HCC. Telomere length and telomeres expression play a key role in the pathogenesis of HCC. Several studies have found telomere shortening in cirrhotic tissue, independently of the etiology of the liver disease (e.g., alcohol abuse, or viral hepatitis), suggesting that this event might represent a hallmark of liver senescence and chronic hepatitis[27-29]. In contrast to cirrhotic tissue, in 44-59% of the HCCs a reactivation of the *TERT* gene is observed[30]. Cellular senescence found in cirrhotic tissue, followed by *TERT* reactivation, is one of the mechanisms that may explain the development and progression of HCC in cirrhosis. In particular, with the accumulation of gene alterations, senescence can induce neoplastic transformation, whereas subsequent telomerase activation can lead to a neoplastic progression (**Figure 1**).

Reactivation of TERT can also be caused by HBV infection (**Figure 1**). HBV is an enveloped virus with partially double-stranded DNA, with the capacity to integrate its own genome into that of the host, leading to the deregulation of the gene involved. *TERT* promoter is the most frequent site of integration (38,5%) in HBV-related cancers and the viral integration leads to *TERT* overexpression[31, 32]. Intriguingly, *TERT* mutations have never been described in hepatocellular adenoma (HCA) differently from *CTNNB1* (see "*CTNNB1* and HCC" paragraph) [13, 32], [33, 34]. *TERT* mutations in HCCs have been statistically correlated with (**Figure 1**):

i) age over 65 years (P = 0.018), HCV infection more than HBV (P = 0.048), and intratumoral morphological heterogeneity (P = 0.0001) [35, 36]. In a study performed on 97 HCCs by Kwa and colleagues, the histological patterns in the tumor areas are classified into four groups: early, well, moderate, and poor[35, 36]. In particular, regarding the morphological aspect, in *TERT* mutated HCC they observed two or more histological patterns as opposed to *TERT* wild-type HCCs, which showed only a single dominant pattern [35];

ii) Alcohol consumption[32, 37]: Schulze and colleagues performed a study on 243 surgically resected HCCs, the 60% of the alcohol-related HCCs had a mutation in the *TERT* gene promoter[32, 37];

iii) *CTNNB1* mutations: several studies have shown the association between *CTNNB1* and *TERT*[38-40]. This correlation was demonstrated for the first time in a mice model in which it was observed that β-Catenin binds the *TERT* promoter and participates in the control of its expression [40].

iv) poor prognosis, (P = 0.041). A study by Oh and colleagues on telomere length in HCC showed that telomere elongation is a poor prognostic factor, as it decreases overall survival (P = 0.044) [41]. Moreover, also in the case of high telomerase activity the prognosis was unfavorable (P = 0.009) [41].

CTNNB1

CTNNB1 gene encodes for β-Catenin, a protein that performs several cellular functions: when interacting with the cadherin protein complex, β-Catenin is important for the stabilization of the cytoskeleton and intracellular adhesions, but it also plays a role as a transcription factor in the canonical Wnt/β-catenin pathway. This pathway is involved in embryonic development, cellular homeostasis, and several diseases[42]. The cytoplasmic concentration of β-Catenin is tightly controlled through its ubiquitination and proteasomal degradation. The phosphorylation required for this degradation mechanism is performed by Glycogen Synthase Kinase 3 alpha and beta (GSK3α and GSK3β), through the action of Axin and the protein adenomatous polyposis coli (APC)[43, 44]. In the cytoplasmic membrane, there are receptors for the Wnt molecules, called Frizzled: the ligand-receptor complex triggers a cascade of cytoplasmic reactions, leading to the activation of the Disheveled protein (DSH). This protein binds Axin, preventing the bond between Axin and GSK3[45]. This mechanism inhibits the proteasomal degradation of β-catenin. Given that *CTNNB1* continues to be transcripted, the β-catenin cytoplasmic concentration increases. Once all the β-catenin cytoplasmic binding sites are saturated, β-catenin protein is translocated into the nucleus. Here β-

catenin interacts with many transcriptional factors, in particular with the T-cell factor (TCF) / lymphoid enhancing factor (LEF), to promote the transcription of target genes, such as *c-Myc*, *CyclinD-1*, and *Jun* (**Figure 2**). Most of these gene targets encode for oncoproteins, leading to the activation of oncogenic mechanisms (e.g., uncontrolled growth or escape from apoptosis)[46]. For this reason, β-catenin is a molecule that may be involved in carcinogenesis and tumor progression of several cancers: HCC, lung cancer, brain and cerebellum cancer, breast cancer, colon cancer, leukemia, and others[47-49].

The Wnt pathway can also be activated by transforming growth factor-β (TGF-β), and dysregulation of its signaling pathway is associated with an invasive phenotype and plays a central role in inflammation, fibrogenesis, and immunomodulation in the HCC microenvironment [50, 51].

Activating mutations found in the *CTNNB1* gene are generally substitutions or in-frame deletions in hotspot regions that encode for the part of the protein that acts as a domain for the *APC/AXIN1/GSK3B* complex. Thus, β-catenin is not degraded by proteosome and then uncontrollably activates the transcription of oncogenes[52].

CTNNB1 and HCC. In HCCs, *CTNNB1* mutations are among the most encountered genetic alterations, with a frequency of 20-40%[32]. Regarding therapy, *CTNNB1* mutations induce resistance to immune checkpoint inhibitors (anti-PD-1/PD-L1 inhibitors and anti-CTLA4)[53]. Another important aspect concerns HCA: according to the literature, 5-10% of HCA are subject to malignant transformation, but the most recent WHO considered CTNNB1 mutated HCAs as a specific subtype, with a higher risk for malignant transformation that could lead to the development of HCC. In HCA mutations in *CTNNB1* are identified in 11-43% [54-56]

CTNNB1 mutations are not the only alterations found in HCCs, regarding Wnt/β-catenin pathway. In fact, also mutations in Axin and APC have been detected, respectively in 6-15% and 2-4% of HCCs [57]. Generally, mutations in *CTNNB1*, Axin, and APC are mutually exclusive[57].

HCCs with mutated *CTNNB1* are statistically correlated to (**Figure 2**):

i) HCV infection[36]. For example, in a study by Huang and colleagues performed a study on 22 HCV-related HCCs, an association between HCV infection and activation of the Wnt signaling pathway caused by the β-catenin mutation was found in 41% of cases [36], while according to WHO, 30% of HCCs caused by HCV harbors a mutation in the CTNNB1 gene.;

ii) Alcohol consumption: Schulze *et al*. studied 243 surgically resected HCCs, and 37% of the alcohol-related HCCs harbored a mutation in the *CTNNB1* gene[37];

iii) *TERT* mutation[38, 39]: correlations between *CTNNB1* and *TERT* have been described in "*TERT* and HCC" paragraph;

iv) *ARID1A* mutations (P = 0.05) and *NFE2L2* mutations (P=0.015) associations with *CTNNB1* were demonstrated by Guichard and colleagues in a study performed on 125 HCCs[57].

TP53

TP53 gene encodes for the p53 protein, which owes its name to its molecular mass (53 kDa). p53 is called "the guardian of the genome" because it is an oncosuppressor that regulates the cell cycle, apoptosis, and genomic stability by preventing genomic mutations. The p53 pathway is crucial in cellular mechanisms as it interacts with other signal transduction pathways (e.g., retinoblastoma pathway, Wnt-beta-catenin, cyclin-cdk). Plenty of positive and negative autoregulatory feedback mechanisms act on p53 functions [58, 59]. The activation of p53 occurs in response to many different stressors, both intrinsic and extrinsic to the cell (e.g., gamma or UV radiation, oxidative stress, osmotic shock), that put faithful duplication of genetic material at risk[60]. The key event for p53 activation is the phosphorylation of the N-terminal domain by protein kinases. This event leads to the accumulation of p53 in the stressed cells, through an increase in the half-life of the protein, and an increase in efficiency as a transcription factor. After this activation, p53 initiates a program that blocks the cell cycle, leads the cell to senescence, and then to apoptosis [61] (**Figure 3**).

In unstressed cells, cytoplasmic levels of p53 are kept in check through its degradation. The Mdm2 protein binds p53, transports it from the nucleus to the cytosol, and acts as a ubiquitin ligase so that ubiquitin binds to p53 Leading to proteasome degradation [62]. If the *TP53* gene is altered, the p53 protein cannot function properly, driving to tumorigenesis and tumor progression. As early as 1990, *TP53* was defined as the most frequently mutated gene in human cancers. *TP53* mutations remain among the most frequent and most significant in more common human cancers, although the frequency of mutations is highly variable depending on the type of cancer: from 90% in the ovary, 50-80% in the lung up to less than 5% in the cervix[63]. Individuals affected by Li Fraumeni syndrome carry a mutated allele of *TP53* and this syndrome predisposes to the development of several types of cancers[64].

TP53 and HCC. Approximately 15-40% of HCCs carry mutations in the *TP53* gene, with a higher frequency in advanced tumors[65]. Intriguingly, a specific *TP53* mutation is significantly associated with dietary intake of aflatoxin B1 (AFB1), a mycotoxin produced by Aspergillus fungi: exposure to AFB1 induces the transversion G → T at the *TP53* codon 249, leading to the p.R249S (c.747G>T) substitution. This mutation could then be considered a mutational signature of exposure to AFB1, in HCC[66-68].

HCCs with mutated *TP53* are statistically correlated to (**Figure 3**):

i) HBV infection and Aflatoxin B1 exposure[68, 69]. Lunn and colleagues conducted a population-based study on 110 HCCs. The relative risk (RR) that they obtained for HBV infection (RR=17.0), Aflatoxin B1 exposure (RR=17.4), and the two risk agents together (RR=67.7), confirmed the correlation between these agents and HCC development [69]. Exposure to aflatoxin B1 induces the p.R249S substitution in the *TP53* gene and HBV infection causes integration of the viral genome into that of the host, promoting mutations in genes crucial for cellular regulation, such as *TP53*. For these reasons HBV infection and Aflatoxin B1 promote a high rate of mutagenesis in HCC[68, 69];

ii) *TP53* alterations were usually exclusive from *CTNNB1* mutations ($P = 0.0001$), but not from *AXIN1* and *APC*[57].

MOLECULAR HCC SUBTYPE CLASSIFICATION

Over the years, many groups have tried to classify HCCs according to a molecular basis, but a univocal classification has been never reached.

Kabashima *et al* grouped different classifications, starting from that of Shimada *et al*, and grouped HCCs into three molecular subtypes (MS1, MS2, MS3). The groups identified by the different studies are only fairly overlapping with each other. These classifications were based on clinical, molecular, and immunological features[70, 71].

Regarding gene alterations, some correlations were found between *TP53* and *CTNNB1*. *TP53*-mutated HCCs were classified into the MS1 group, which correlated with unfavorable prognosis, viral infection, high serum AFP levels, vascular invasion and proliferation, extensive mitotic activity resulting in chromosomal instability, and stem cell-like properties.

CTNNB1-mutated HCCs were placed into the MS2 group, correlating with aberrant activation of Wnt/ β-Catenin pathway, which could explain the high rate of methylation in CpG islands present in this group, as the constitutively active β-catenin protein recruits a DNMT1 methyltransferase. Another feature associated with the MS2 group is the immunosuppressive phenotype and, moreover, this class is considered non-proliferative/Less progressive than others.

The MS3 group is not associated with molecular signatures, but only with metabolic disease-associated tumors[51, 72-74].

The *TERT* gene is rarely found in these classifications. However, it should be considered that *TERT* most frequent mutations fall in a promoter region usually not covered by the exome sequencing studies. The TCGA 2017 classification detected *TERT* mutations in HCC and includes HCC-TERT samples in iCluster2 and iCluster3 [17]. In 2019, further classification was drafted by Yang and colleagues that divides HCCs into 3 groups (C1, C2, C3) [75], overlapping with a previous one performed by Hoshida and colleagues [51]: C1–S3, C2–S1, C3–S2.

MOLECULAR HCC SUBTYPES AND PATHOLOGICAL FEATURES

In 2017, Calderaro and colleagues discriminated HCCs based on the presence of *TP53* or *CTNNB1* mutations, considering that these are two genes that mutate in a mutually exclusive manner and together comprise 57% of HCCs[76]. In this study, CTNNB1-mutated HCCs were described as larger than the CTNNB1-WT HCCs, but characterized by a lower tumor grade, with microtrabecular and pseudoglandular patterns of growth, without inflammatory infiltrates and with the presence of cholestasis[76]. Conversely, TP53-mutated HCCs were described as poorly differentiated tumors, with large multinucleated and pleomorphic cells, solid pattern of growth, frequent vascular invasion, and angiogenesis[76].

Selected subtypes of HCCs recognized by the most recent WHO Classification of Tumors were described to have specific molecular alterations (**Figure 4-5**) [14]:

i) up to 63% of *Steatohepatitic HCCs* (SH-HCCs) were associated with NAFLD. This type of HCC is characterized by the following histologic features: macrovesicular steatosis, Mallory-Denk bodies, ballooning of tumoral hepatocytes, inflammation, and trabecular or pericellular fibrosis. With regard to key molecular features, SH-HCCs were significantly associated with a lower frequency of *CTNNB1* mutations, higher rate of mutations in *TERT* and *TP53*, IL-6/JAK/STAT activation, high level of C-reactive protein, and serum amyloid A positive at immunohistochemistry[14, 77-80].

ii) *Clear cell HCCs* are considered a well-differentiated type, characterized by a cytoplasmic clearing, due to accumulation of glycogen, lipopolysaccharides, mucopolysaccharides, or cytoplasmic vesicles. IDH mutations were identified in 25% of clear cells HCC, and these alterations were significantly associated with a worse prognosis. Moreover, *IDH* mutation is also found in intrahepatic cholangiocarcinoma, a tumor with a significantly worse prognosis than HCC[14, 80-83].

iii) *Macrotrabecular massive HCCs* (MTM-HCC) are frequently larger than 50 mm with vascular invasion, correlated high Alpha-fetoprotein (AFP) serum levels, high expression of angiopoietin 2 (Ang2), and vascular endothelial growth factor A (VEGFA). At the histological level, this subgroup is characterized by massive trabeculae surrounded by vascular spaces and coated by immature endothelial cells. On the

molecular side, *TP53* mutations and *FGF19* amplifications have been detected. MTM-HCC is an aggressive phenotype associated with a worse prognosis[14, 80, 84, 85].

iv) *Scirrhous HCCs* (S-HCCs) develop in the non-cirrhotic liver, and they are characterized by hyaline stroma, intratumoral fibrosis with thin trabecular pattern growth (due to this characteristic is easily confusable radiologically to cholangiocarcinoma), or the lymphoepithelioma-like subtype, consisting of dense intratumor lymphocytic infiltration. S-HCCs may exhibit *TSC1/TSC2* mutations and TGF-beta signaling activation. Regarding prognosis, S-HCCs are an aggressive subgroup, often with invasion of the portal vein, but as far as long-term follow-up is concerned, the prognosis is similar or sometimes better to conventional HCCs[76, 86, 87].

EXPERIENCE FROM OUR CENTER

Our preliminary results focused on 36 prospectively enrolled patients, all resected for HCC and selected for Next-Generation Sequencing (NGS) by means of a laboratory-developed multi-gene panel Gene-Studio S5 sequencer, which comprises also specific target regions, including *TERT*[15]. We detected single mutations in *TERT* promoter in 7 (19.4%) cases, in *TP53* in 4 (11.1%), in *CTNNB1* in 2 (5.6%). *TERT* and *CTNNB1* coexistent mutations were observed in 8 (22.2%) cases, while *TERT* and *TP53* were in 8 (22.2%). In 7 (19.4%) cases no one of these three genes, or other, was detected as mutated (**Figure 6**).

In line with a previous study by Calderaro *et al*[76], we observed a trend of *TERT*-mutated HCC towards a macrotrabecolar or solid architecture. Moreover, the presence of *TERT* promoter mutations in combination with *TP53* mutation correlated with high-grade HCC (p=.011; **Figure 7**).

Interestingly, no correlations were found between mutations and tumor dimensions. This evidence leads us to hypothesize that the presence of *TERT* promoter mutations, alone or in combination with *TP53* alteration, correlates with a morphological progression in HCC, in terms of a higher tumor grade and an architecture more related to aggressive behavior (solid, macrotrabecular), but not of a dimensional evolution.

Most of the HCC in non-cirrhotic livers of our series showed no mutations or harbored only a *CTNNB1* mutation (P = 0.031), as a countercheck of the correlation between tumor progression and mutations. The validation of these results on a larger series as well as with post-surgical follow-up might indicate that small HCC may have an aggressive behavior from a molecular and morphological point of view, despite their dimensions.

CONCLUSION

The molecular signature of a tumor is becoming increasingly important in the approach of patients with different types of cancers, on diagnostic, prognostic, and predictive grounds. In the age of precision medicine, the study of HCC mutations is still a field that worth to be investigated. Considering both the literature data and our personal experience, about 80% of HCC harbor mutations in at least one among *TERT*, *TP53*, or *CTNNB1* genes, with different biological and clinical implications.

In the next future, a deeper analysis of these three genes is surely desirable, since a molecular characterization of HCC would open to personalized therapies, as happened for other cancers (e.g., lung adenocarcinomas, melanomas, gastrointestinal stromal tumors, colorectal adenocarcinomas). Moreover, the evidence of a tight correlation between the mutational profile and the HCC morphology is likely to imply an increasing integrate approach between anatomic pathology and molecular laboratories.

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1	F. Vasuri, T. Maloberti, M. Riefolo, G. Germinario, V. Sanza, M. Ravaioli, M. Cescon, D. de Biase, A. D'Errico. "Morphological features and biological aggressiveness of hepatocellular carcinomas with TERT and TP53 mutations. Preliminary monocentric results on resected patients", Digestive and Liver Disease, 2022 Crossref	88 words — 2%
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