Chapter

Hepatic Progression of Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma constitutes an ongoing challenge due to its incidence and the high mortality related to it. Metastases and relapses even after treatment with curative intent are frequent. The liver is a common site for metastasis because of anatomical and physiological reasons; its position, the particular cytoarchitecture and cell populations, and its peculiar immunologic properties make it a favorable and tolerogenic environment; the inflammatory state with the alteration of the cytoarchitecture and of the microcirculation associated, and gut permeability and metabolic diseases cause the development of a liable site to progression of hepatocellular carcinoma. The difficulty of always having an early diagnosis and the lack of therapeutic flow charts including the biological behavior of the disease have always posed great difficulties in dealing with it. In the last few years, mechanisms involved in the onset and in the progression of hepatocellular carcinoma are a source of great interest; the discovery of pro-neoplastic and pro-metastatic conditions, of the cross talk between organs and cells, of progression pathways, of mediators contributing to proliferation and metastasis and of modular check points, of miRNAs, all potential therapeutic targets, appear promising for transforming the approach to hepatocarcinoma, offering the possibility of earlier diagnosis, customizable treatments, and better outcome.

Keywords: hepatocellular carcinoma, liver metastasis, hepatic progression of hepatocellular carcinoma, immunotolerance, ischemia/riperfusion injury, miRNAs

1. Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death. Locoregional treatment, surgical resection, and liver transplantation are main and most efficient treatments, but the risks of recurrence and metastasis are very high (70% after primary liver resection with curative intent) [1].

The risk of recurrence in HCC is high because of the biological and morphological nature of the liver.

Recurrent disease includes both intrahepatic metastasis, which usually form within the first 2 years of diagnosis, and de novo cancer that generally occurs later. The hepatic vascular anatomy and immunological characteristics create a

pro-neoplastic niche for metastasis, while the continuing damage to the liver creates pro-neoplastic sites susceptible to secondary tumors. Liver metastasis represents a big challenge in the battle against cancer because they have a high mortality rate. Around 30–70% of patients die with liver metastasis, and metastasis is responsible for more than 90% cancer mortality [1].

The position of liver, near the gastrointestinal tract, only partially justifies the metastasis toward it. In fact, for other types of "distant" cancer, the liver is the first and sometimes even the only target of metastasis (e.g., uveal melanoma and triple-negative breast cancer). This consequently suggests that the criterion of proximity is only partially a predisposing factor but rather that there are specific tumor and extra tumor elements that predispose to metastasization to the liver. Another factor that has emerged in recent years is the exchange of information and the "preconditioning" of the liver that occurs before the actual establishment of metastatic colonies.

However, the development of knowledge on the local and systemic factors that promote the advance of the neoplastic disease and favor its metastasis has thus highlighted multiple and rich pathways that are fascinating from a speculative point of view but above all promising for the development of future preventive and therapeutic strategies.

2. Peculiar liver characteristics

The liver is the only organ with double arterial and venous vascularization. It constitutes the first filter for all effluent blood from the gastrointestinal district. The cytoarchitecture with the lobular arrangement, the portal spaces, and the intricate network of discontinuous capillaries (sinusoids) constitutes a unique feature. Multiple cell populations with their pathophysiological implications are responsible of a myriad of actions including favoring or not, the development of metastases.

The cellular structure of the liver is composed of 70% hepatocytes and 30% from non-parenchymal cells, which are crucial in the activation of many very characteristic liver phenomena.

2.1 Hepatic stellate cells

In a physiological condition, the stellate cells represent 1.5% of the entire hepatic parenchyma and 1/3 of the non-parenchymal cell compartment. They have multiple functions, from the ability to store lipids and vitamin A, to their involvement in the reparative phenomena of the hepatic parenchyma, fibrinogenesis, and the maintenance of homeostasis. They have intrahepatic and subendothelial cytoplasmic extensions through which they can establish connections with hepatocytes; are sensitive to the chemotactic stimulus and respond to alpha adrenergic activation. They are activated following the inflammatory stimulus, losing their lipid vacuoles, and developing microfilaments evolving into myofibroblasts. They play a key role in fetal hepatic development, become fundamental in the phenomena of hepatic regeneration of the adult liver and in the remodeling of the extracellular matrix through the secretion of angiogenetic factors, cytokines (IL-6, IL-10, TGF-β), adhesion molecules, connective tissue growth factor (CTGF), Endothelin-1, contributing to the activation of endothelial cells and facilitating the extravasation of neoplastic cells in the liver and supporting metastasizing [1].

They can undergo the mesenchymal-to-epithelial transition and thus become liver progenitor cells giving rise to hepatocytes. They also play an important action at the immunological level as they can act as antigen receptor cells (APCs), receive stimuli from leukocytes, support leukocyte recruitment and activation, modulate the intensity of the activation of the immune response. They are the main cell type involved in fibrogenesis in response to liver damage [2].

2.2 Kupffer cells (KCs)

They represent 20–30% of non-parenchymal cells. They are liver-derived macrophages that support both innate and adaptive immune responses to pathogens originating from the gastrointestinal tract through the effluent blood arriving via the portal vein. They are activated through two types of polarization, classical (M1), when stimulated by IFN, TNF, GM-CSF, or mycrobic stimuli or alternative (M2) when stimulated by IL-4, IL-13 and IL-33. They perform important functions in the mechanism of liver damage, in ischemic/reperfusion injury, in alcoholic liver disease, in nonalcoholic fatty liver disease, in immunotolerance phenomena after liver transplantation, and in endotoxin tolerance modulation. In response to the inflammatory stimulus, they are involved in the activation of HSCs, thus promoting fibrosis through the release of mediators (TGF-beta and platelet-derived growth factor).

Hepatic sinusoid stromal cells, hepatic sinusoidal endothelial cells, and stellate cells are the first liver cells to come into contact with systemic antigens and derived from the gastrointestinal tract [1, 3, 4].

2.3 Immunotolerance

Another fundamental aspect is its function as a tolerogenic immunosuppressive organ toward the molecules absorbed by the intestine and therefore, at the same time, the tumor cells and infectious agents. The liver contains numerous immune system cells, both adaptive and innate, specialized in the recognition and capture of infectious agents with the recruitment of inflammatory and leukocyte cells and the presentation of antigens to lymphocytes in the bloodstream. However, this mechanism must be finely controlled so that the immune response is not implemented toward non-pathogenic molecules such as those of the host or deriving from ingested food. It is this balance between activation of the immune response and its inhibition characterizes the liver as the main organ of the host's first immune barrier [5–7].

The idea that the immune system plays an important role in the development of neoplastic pathology is dated and has been controversial for years; however, several studies on transplants have expanded knowledge about tolerance since the ability to induce tolerance toward the transplanted organ is essential for the survival of the graft.

While it is clear that through the oral route and the gastrointestinal system tolerance can be induced through the local activation of the gastrointestinal immune system, it is, however, shown that much of the induction of the tolerance process takes place in the liver. In particular, survival of the graft in liver transplantation has been demonstrated in subjects without kinship ties of some animal species without immunosuppression and still in some cases in which the expression of a tolerant phenotype allowed the suspension of immunosuppressive therapy [8, 9].

The hepatic parenchyma therefore has very important immunological functions and the peculiar ability to induce tolerance: the activation of the liver immune system, consisting of macrophages, dendritic cells, natural killer, and T lymphocytes, produces immunological mediators (in particular IL 10, TGF beta, and others), which, on the one hand, induce localized immunological suppression aimed at minimizing liver damage, on the other create a tolerant environment for the colonization of metastatic clones. Both sinusoidal endothelial cells and stellate cells activated by TGF-beta and PDGF are strongly involved in the maintenance of local and systemic tolerance phenomena. In addition, the same T lymphocytes made tolerant are then able to return to the primary tumor and suppress the local defense systems [6–12].

The involvement of the immune response and immunocompetence in the development of neoplastic diseases has been, and still is, a subject of debate, because many of the tumor antigens are self-antigens for which the immunological response is weaker and more difficult to measure. However, it is well known that individuals with a decline in immune defenses related to old age, or undergoing immunosuppressive therapies, or suffering from immune system disorders such as chronic infections or autoimmune diseases have an increased risk for the development of metaplasia; even the most common and recognized risk factors for cancers such as smoking, alcohol abuse, advanced age, and poor nutritional status are associated with more or less pronounced alterations of the immune system and risk of metastasis [13].

2.4 Cancer-derived microvescicles

Cancer-derived microvesicles have recently been identified as responsible for very important roles at the level of the tumor microenvironment, contributing through their load to the development of metastases and to the selection of organotropism. The microvesicles produced by the tumor cells are released into the circulation before the cancer cells reach metastatic sites.

The information transmitted by them is able to initiate a congenial soil phenomenon (as claimed in Paget's theory) starting processes of angiogenesis, cancer-associated fibroblasts (CAFs) formation, endothelial cell migration.

The signal mediated by microvesicles allows an exchange of intracellular information with the possibility of modulating the migratory and metastatic behavior of more quiescent cells but also of inducing drug resistance. They are also able to reduce the immune response by increasing the development of immunosuppressive cell populations (PD-1 positive nonclassical monocyte) reprogram the cellular metabolism of neoplastic cells, which is a fundamental step in the extravasation and dissemination of tumor cells.

In the studies of the last few years, it appears increasingly evident that the target organs for the development of metastases undergo phenomena that define their receptivity and mediated by the primary tumor well before the development of metastases.

At the hepatic level, the extracellular vesicles produced by the neoplastic cells first interface with the Kupffer cells. The information deriving from the EVs affects the hepatic stromal cells with the consequent activation of hepatocyte growth factor, which favors the development of fertile soil for the development of metastases [14–17].

3. Metastasis mechanisms, seed and soil theory

The establishment of metastatic colonies requires a complex network of interactions with the microenvironment consisting of extracellular matrix, stromal and inflammatory cells, vessels.

The first and well-known theory on the mechanisms of development of metastases dates back more than a century with the beautiful metaphor of Paget in his concept of seed and soil theory. In a study resulting from the metastatic spread of breast cancer, he compared the tumor to a plant that spreads its seeds everywhere; however, the seeds can germinate and grow only where they find the suitable soil.

Subsequently, other scholars have tried to explain the phenomenon by attributing its origin mainly to anatomical and hemodynamic factors (p.e. J.Edwing). With current knowledge, this provides only a small piece of the complex phenomenon of metastatic advancement and organotropism. We know that there are specific factors of the tumor but also systemic and specific factors of the target organs that determine the evolution of the neoplastic disease. The study of these phenomena is extremely current and promising since metastatic cancer continues to support a very large percentage of deaths and therefore remains an open challenge of fundamental importance [18]. Local phenomena, systemic phenomena, and specific target organ phenomena that are responsible for the advancement and spread of the disease have been identified. Starting from the local phenomena that are characteristic of the neoplastic and peri-neoplastic tissue (but in reality also involved at the level of distant organs site of metastases), we consider the metabolic alterations and the consequent reduction of pH. In fact, it is now a historical acquisition, the condition for which neoplastic cells use a huge amount of glucose to maintain their proliferation generating a large amount of lactic acid as a waste product. This in turn leads to a decrease in pH in the extracellular space with the onset of a condition of acidosis in the tumor microenvironment. This is known as the Warburg effect.

If acidosis by itself favors processes such as metastasis, angiogenesis, and immunosuppression, and given the coexistence of areas with different tumor metabolism (areas closer to the vessels, more oxygenated and farther, more hypoxic), the high concentration of lactates allows phenomena of metabolic adaptation such as "metabolic symbiosis" and "reverse Warburg effect," improving the survival of the neoplastic cells [19].

The local immunological response (cancer immunoediting) plays another fundamental function in allowing neoplastic progression to metastatic disease and is also closely linked to local metabolic and hypoxic phenomena. This concept has appeared of great interest in recent years in view of the therapeutic repercussions that are due to immunotherapy introduction.

This phenomenon is summarized in three steps: the elimination (the first step) of the neoplastic cells by the immune system, the balance (the second step), that is, the moment in which the two systems (neoplasm and organism) are apparently balanced, and finally, the tumor escape when a situation is established in which the immunological response is overcome [20]. Some chemokines, produced in response to inflammatory stimuli of the peritumoral zone, attract leukocytes (polymorphonuclear neutrophils) to the sites of inflammation and play an important role in the homing and proliferation of cancer cells, representing an important component of the phenomenon of escape from the unfavorable environment of

primitive cancer cells. However, the classic concept of peri-neoplastic inflammatory tissue with pro-tumoral activities has undergone conceptual evolutions with the definition of an immunological threshold beyond which the normally tumorigenic phenomenon becomes rather beneficial by developing the idea of an intensification of the local inflammatory stimulus can be exploited in therapeutic terms [10–12, 21].

4. Liver diseases and hepatocellular carcinoma progression

The process of metastasis is a complex phenomenon that occurs through multiple steps, from intravasation after the escape from the primary tumor, to the overcoming of the systems of recognition and cellular destruction, to the invasion and survival in the blood stream. Cells that manage to overcome these steps have developed a high capacity for metastasis through accumulation of genetic and epigenetic alterations including microRNA (miRNA) expression changes.

In HCC, venous metastases develop through dissemination by portal and through the formation of neoplastic thrombi by neoplastic cells that have acquired the molecular changes that allow them to survive and invade the venous stream [22].

Hypoxia is common to many tumors. In HCC, hypoxia is present and responsible of progression and metastatization.

Liver cirrhosis and the rapid growth of the neoplastic nodule determine a reduction in blood flow with the consequent establishment of a hypoxic state. Both liver cirrhosis and tumor size (>8 cm) are independent risk factors for development of portal vein tumor thrombi. PVTT is present in 20–70% of HCC and correlates with poor prognosis.

Under hypoxia conditions, the expression of 14-3-3ζ is increased, which induced hypoxia-induced factor-1α (HIF-1α) expression by stabilizing HIF-1α protein. This resulted in an enhanced EMT response of HCC cells, promoting the formation of PVTT and HCC metastasis.

Both HIF-1α levels and PVTT formation in HCC are strongly correlated with 14-3-3ζ expression [23].

During the metastasis process, the biological characteristics of the target organs are decisive. Generally, the target organs, already in the initial state of the disease, even before the onset of metastases, have already been affected by factors deriving from the primary tumor. Furthermore, the metastatic microenvironment does not depend only on the anatomy and biology of the target organ but also on the pathophysiological phenomena altered by the products of neoplastic cells or by preexisting conditions. Numerous studies have evaluated the implications of a preexisting liver disease or chronic inflammatory condition on the evolution of the development of metastases with sometimes conflicting results [24–28]. The coexistence of a preexisting inflammatory activation, the presence of a subversion of the parenchymal structure, an alteration of the extracellular matrix, the activation of stellate and Kupffer cells, and the condition of oxidative stress that is created are phenomena that are recognized responsible in the promotion of metastasis [1, 29–32].

Given its double vascularization with a much higher venous supply, it is mainly a hypoxic microenvironment [33].

If on the one hand, the frequent metastasis to the liver depends on its peculiar characteristics of the microcirculation and sinusoidal permeability and on the

physiologically immunotolerant environment that distinguishes it, on the other hand, the neoplastic cells to constitute metastatic colonies enter into "metabolic" competition with normal liver cells precisely because of the hypoxic environment and learn to "mimic" the metabolic behavior of normal liver cells.

Recent studies have demonstrated the ability of an epigenetic remodeling, which, through enhancers or super enhancers, can modify the specific transcription program of circulating tumor cells (specific to the tumor type) by making them acquire a liver-specific transcription program [34].

The molecular mechanisms that metastatic cells have acquired to obviate the metabolic problems deriving from the hypoxic environment, that is, different according to the tumor type, have also been described.

The activation of intrahepatic cells can modify the metabolic behavior of metastatic cells. In particular, stellate cells, normally quiescent, when activated in a context that from poorly inflammatory becomes inflammatory can affect their metabolic state, proliferation ability, and stem cell characteristics.

Also ketone bodies metabolism, enterohepatic circulation of bile acid, and ammonia metabolism may change the metabolic behavior of cancer cells, since neoplastic cells are also able to use waste products that hepatocytes cannot use, to generate energy, thanks to their marked ability to adapt, confirming how their metabolism is anything but a static phenomenon. These alternative pathways are therefore also able to determine activation of quiescientious cells [35].

However, it is essential to consider the complexity, bidirectionality, and specificity of this whole cascade of events. In fact, the systemic repercussions, which occur from the beginning of the onset of neoplastic pathology, presuppose a two-way communication between neoplasm and host with the establishment of both protumor and antitumor pathways; moreover they are patient-specific, dependent on the specific patient's background and on the coexistence of different pathophysiological conditions including comorbidities and ongoing therapy; similarly the metastatic potential of circulating cancer cells is extremely heterogeneous from patient to patient.

4.1 Ischemia/reperfusion injury, gut-liver axis, and angiogenesis

Ischemia/reperfusion damage (IRI) is a well-known para-physiological phenomenon that follows hepatic surgical resection and transplantation procedures. It's is commonly interpreted as a state of sterile inflammation. After ischemic state is established, the liver tissue initiates a cascade of events leading to hepatocellular injury, alteration of liver function, and worsened oncological outcomes in the presence of cancer [36].

After a time of iatrogenic ischemia induced by clamping the portal peduncle to limit blood losses or necessary for the packaging of vascular anastomoses, the restoration of perfusion causes a cascade of inflammatory and repair phenomena.

If during the ischemia phase, the cells are subjected to hypoxic stress with a decrease in pH, ATP depletion, accumulation of intracellular Ca, and activation of various forms of cellular death, then with the reperfusion phase there is the formation of reactive oxygen species (ROS), activation of the immunological response, release of chemokines and inflammatory cytokines, of cell damage mediating molecules (DAMPs), and activation of hepatic cellular subpopulations.

In this circumstance, through the phenomenon of immune escape and neo-angiogenesis promoted by the cascade of events resulting from IRI, the risk of recurrence and disease progression makes its way.

At the same time, the portal clamping causes a venous congestion of the gastrointestinal tract with hypoperfusion and subsequent establishment of mucosal damage that determines an alteration of permeability with subsequent bacterial translocation. Ischemia/reperfusion damage and increased intestinal permeability are related to a risk of recurrence both after transplantation and after liver resection due to the onset of exacerbated inflammation.

Therefore, the modulation of the inflammatory response and the modulation of the gut-liver axis prove to be key points on which to act to reduce the oncological risk.

Steatotic livers are more sensitive to damage from ischemia/reperfusion due to alterations in the microcirculation caused by the accumulation of lipids with a consequent decrease in the sinusoidal space and because of a lower amount of stored energy and a greater sensitivity of the cell membrane to lipid peroxidation caused by ischemia/reperfusion. The same applies to aged livers: are much more sensitive to ischemia/reperfusion damage [37].

Recently it has been observed that small for size syndrome is also involved as a negative prognostic factor after liver surgery in terms of risk of recurrence for HCC. It constitutes a postsurgical complication with hepatic insufficiency and due to its analogy with the damage induced by the phenomenon of ischemia/reperfusion, it also causes mechanical damage in the acute phase, in fact, highlighting a correspondence between parenchymal liver damage and the possibility of implantation of circulating neoplastic cells.

Moreover, it has been seen that these acute-phase phenomena not only favor the implantation of neoplastic cells for factors related to the other microcirculation and the inflammatory cascade but also are able to modify the behavior of neoplastic cells favoring their aggressiveness by directly activating cell migration and invasion pathways [37].

Promising strategies to reduce this risk are normothermic or hypothermic oxygenated perfusions aimed at reducing oxidative stress especially when the graft is suboptimal and is therefore even more sensitive to the cascade of inflammatory phenomena triggered by IRI.

Changes in iatrogenically induced ischemia during hepatic resection have also been explored in order to reduce intraoperative bleeding such as selective portal clamping, maintaining arterial flow, and remote ischemic preconditioning. These strategies appear to reduce ischemia/reperfusion damage resulting in a positive effect in terms of the risk of HCC recurrence [38].

4.2 Renin-angiotensin system

The renin-angiotensin system seems to be involved in multiple aspects of the evolution of HCC and in the development of metastases. Overexpressions of components of this axis in hepatocarcinoma have been highlighted. In addition, its components are able to promote cell proliferation, angiogenesis, extracellular matrix formation, and fibrosis progression; they can interact with the m-TOR pathway and inhibit apoptosis.

They promote fibrosis: some components of the renin-angiotensin axis are able to modulate liver fibrosis through the activation of HSCs and the deposition of extracellular matrix; treatment with Losartan as a pharmacological agent active on angiotensin II receptors is able to reduce liver fibrosis.

They stimulate neo-angiogenesis by modulation of growth factors such as VEGF and TGF-beta, promoting epithelium-mesenchymal transition, inducing the formation of reactive oxygen species (classical pathway) or through the activation of non-classical TGF-beta/MAPK pathway involvement of apoptotic, metabolic, and cell proliferation phenomena [39].

The mechanisms involved are:

- Activation of AT1R promoting cell proliferation, inflammation, angiogenesis, and extracellular matrix formation while Mas receptor (MasR), other component of RAS, has a protective role since it inhibits the effects of AT1R.

- Phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway promotes HCC cell proliferation and inhibits apoptosis, and the RAS system reduces the survival rate of HCC patients by mediating PI3K/AKT/mTOR pathway.

- Ang II induces vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF-β) to promote angiogenesis and aggravate liver fibrosis, respectively.

4.3 Insulin resistance and diabetes type 2

Insulin resistance and diabetes type 2 are involved in the onset and progression of hepatocellular carcinoma.

In chronic liver disease generally underlying the onset of HCC, there are conditions of insulin resistance and type 2 diabetes linked to obesity and dyslipidemia but also diabetes of hepatic origin caused by HCV, alcohol intake, structural alterations, reduction in liver mass with consequent reduction of insulin clearance by Kupffer cells and endothelial cells or by constitution of collateral circles following portal hypertension with the establishment of shunts and therefore bypassing the hepatic extraction.

All these conditions lead to increased blood insulin levels. An excess of insulin in blood dictated by resistance to it determines secretion at the hepatic level of insulin-like growth factor (known to stimulate cellular proliferation and also inhibit apoptosis within the liver) [40].

A situation of insulin resistance is established following hyperinsulinemia due to the consequent downregulation of IRs, i.e., reduced receptor affinity, reduced availability at target, and a reduced efficacy, due to increase in glucagon, growth hormone, IGF, free fatty acids, and cytokines [41, 42].

Diabetes mellitus (DM) is identified as a negative prognostic indicator in hepatocellular carcinoma (HCC) since it has been shown to be associated with significantly higher incidence of histological macrovascular invasion and a higher rate of distant metastatic disease [43].

4.4 Cancer stem cells, down- or upregulated pathways, driving genes and epigenetic modifications

During the inflammatory and regenerative state that determines the development and progression of hepatocarcinoma, many events take place including the expansion of stem cells, the modification of the microenvironment as well as the multiple genetic and epigenetic changes that give the neoplastic cells the ability to survive and proliferate. Organogenesis and the development of a hepatic neoplasm are similar phenomena.

In both cases, cell proliferation, angiogenesis, and cross talk phenomena with the microenvironment occur.

Cancer stem cells are tumorigenic and metastatic and are markedly elevated in chronic liver diseases; hepatocytes, in these conditions have lost part of their proliferative capacity. This causes the expansion of stem cells (ductular reactions). The

self-renewal, differentiation, proliferation, survival, angiogenesis, and migration of CSCs in several malignancies are promoted by the Notch signaling pathway.

To obtain tumor regression, eradication of cancer stem cells is considered sufficient. This has an important implication from the point of view of therapeutic application as the eradication of cancer stem cells could determine the regression of the neoplasm [44].

The acquisition of changes in the upregulation or downregulation of progression pathways (EGFR, Ras/Raf/Erk, PI3/Akt/mTOR, JAK, Shp2,..) is a phenomenon that allows the progression of the disease and that determines the development of resistance or poor efficacy of pharmacological therapies.

This suggests on the one hand the need to identify the type of HCC on the basis of the expression of these alterations as it results in a prognostic stratification based on the expected response to therapy, and on the other hand, it offers the possibility of studying personalized therapeutic combinations aimed at improving the outcome by acting on the molecular path that determines the response.

The introduction of therapies for advanced HCC not susceptible to surgical therapy (multikinase inhibitors) has made it possible to expand the therapeutic possibilities thanks to the discovery of new therapeutic targets but also has shown a great heterogenicity of response and high levels of resistances that require customization therapeutic strategies [45].

Tumorigenesis and tumor progression are promoted by genetic and epigenetic changes. This makes it possible to overcome immunological barriers, to survive changes in the environment, pH, and metabolism, to acquire the ability to metastasize and to acquire resistance to therapies.

Alterations that commonly occur in HCC have been highlighted:

- CTNNB1-related WNT-beta-catenin and most commonly present in HCV-related liver disease;

- VEGFA and IGF2, which promote neoplastic progression and angiogenesis.

- KRAS, quite infrequent in HCC but closely related to MKI resistance.

Each of these alterations constitutes a possible therapeutic window; the inhibition of Wnt/β-catenin pathway determines a reduction in the phenomenon of epithelial mesenchymal transition and increases radiosensitivity.

Several specific molecular targets for antiangiogenic agents are being explored; sorafenib, exerts antiangiogenic effects through VEGF receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) inhibition [46].

4.5 miRNA

MicroRNAs are small single-strand non-coding of about 22 nucleotides in length.

MicroRNAs binds to complementary sequences of target mRNA and performs the posttrascriptional regulatory activity; each miRNA can target hundreds of mRNA.

They are important regulatory molecules in gene expression processes. miRNAs are involved in physiological and pathological processes and play different roles in carcinogenesis processes, progression, invasion, metastatization, cell cycle, apoptosis, and drug resistance. Some miRNAs are involved in modulating epithelial-mesenchymal transition through downregulation of E-cadherin, enhancing HCC metastasis, and some are involved in PVTT formation determining high level of TGF beta and favoring immune escape.

They have a dual role: in HCC some miRNAs are overexpressed, others are down-regulated, suggesting they can act as oncogenic factor or as tumor suppressor.

microRNAs also play pivotal roles in immune-modulation and antitumor immunity [47–50].

The cross talk between epigenetics and miRNA is important in the molecular pathogenesis of HCC: some studies demonstrate that epigenetic alteration can silence miRNAs with tumor suppressor activities.

Restoring the expression of tumor suppressor miRNA could be used for cancer treatment [51].

The molecular heterogeneity of HCC indeed still represents a critical factor; some microRNAs are associated with HCC or related to HCC subtypes, suggesting the potential role of microRNAs for HCC patient stratification terms of diagnosis and prognosis but also in the allocation of the best therapeutic plan with the potential for personalized adjuvant therapy; miRNAs are promising tools also as molecular biomarkers (both tissue and circulating) to predict mestastasis and postsurgical recurrence as well as therapeutic targets [52].

From the therapeutic point of view, the possibility to use miRNA looks very promising: there are studies on the silencing of "oncogenic" miRNAs through the endovenous administration of antagonists, on miRNA restoration, of those with onco-suppressive function, through administration by adeno-associated virus or non-viral miRNA delivery system [52].

5. Conclusions

The treatment of HCC poses a great challenge due to the high incidence, high mortality rate caused by relapses after treatment. For years many classifications have followed one another in order to try to standardize the treatment based on the stage of the disease to obtain the best possible benefit. However, an insufficient definition of the stratification caused by the heterogeneity of the biological behavior and also of the patient's response has always emerged. The absence of biomarkers indicative of the degree of aggression and the biological features has always placed a major limitation causing "blind spots" at the time of therapeutic choice.

Furthermore, due to the very nature of the disease and the general conditions of the patient, the diagnosis is often made late or the surgical possibilities are not feasible. The study and discovery of the steps of carcinogenesis and of the phenomena involved in the progression of the disease are of great interest and have provided fundamental elements for the treatment (with the introduction of new therapeutic substances starting from MK inhibitors to miRNA or immunoterapies).

Thanks to the discovery of pathways and checkpoints, essential steps for the rise and progression of HCC, many therapeutic strategies have been studied with the aim of blocking a certain "step" or even reprogramming the aggression and the ability to metastasize or to modulate the resistance/sensitivity to therapies. The introduction of biomarkers and diagnostic strategies such as liquid biopsy allows to carry out diagnostic studies in much earlier stages than in the past, overcoming problems of this disease in terms of seeding risk, inadequacy in describing phenomena of vascular invasion and biological aggression, or to dynamically monitor neoplastic progression.

The combination of these diagnostic tools and these therapeutic strategies seems extremely promising; they could substantially change the approach to HCC with early diagnosis and patient-tailored therapies.

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