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Mini-review

Tetraspanin-enriched microdomains and hepatocellular carcinoma progression

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ABSTRACT

As in many tumors, heterogeneity within the cell population is one of the main features of hepatocellular carcinoma (HCC). Heterogeneity results from the ability of tumor to produce multiple subpopulations of cells with diverse genetic, biochemical and immunological characteristics. Little is known about how heterogeneity emerges and how it is maintained. Fluctuations in single cells can be masked or completely misrepresented when cell populations are analyzed. It has become exceedingly apparent that the utility of measurement based on the analysis of bulk specimens is limited by intra-tumor genetic and epigenetic heterogeneity, as characteristics of the most abundant cell type might not necessarily predict the properties of cell populations. Yet, such non-uniformities often unveil molecular patterns that can represent mechanisms of tumor progression. Interestingly, variability among single cells in a population may arise from different responses to intrinsic and extrinsic perturbations mainly mediated by the plasma membrane. The association of certain proteins, including tetraspanins, and lipids in specific location on the plasma membrane constitutes specialized structure called tetraspanin-enriched microdomains (TEMs). TEMs organization in cancer may reveal essential clues for understanding pathogenic mechanisms underlying cancer progression. Along these lines, TEMs and HCC progression represent a valuable paradigm for gaining a deeper understanding of such mechanisms.

Introduction

Hepatocellular carcinoma (HCC) is responsible for 85% of primary liver malignancy with high mortality rate worldwide [1]. Several factors including geographical region, gender, racial and ethnical variation can affect the incidence of HCC. Briefly, sub-Sahara Africa and eastern Asia are documented as the regions with high incidence of HCC. In contrast, America, north Europe and Oceania are considered as low-rate [2,3].

Major risk factors for hepatocellular carcinoma include infection with HBV or HCV, alcoholic liver disease, and probably nonalcoholic fatty liver disease. Less common causes include hereditary hemochromatosis, alpha1-antitrypsin deficiency, autoimmune hepatitis, some porphyrias, and Wilson's disease. The distribution of these risk factors among patients with hepatocellular carcinoma is highly variable, depending on geographic region and race or ethnic group [4–9]. The most part of these risk factors lead to the formation and progression of cirrhosis, which is present in 80–90% of patients with hepatocellular carcinoma. Cirrhosis from any cause predisposes to HCC and hence can be considered a premalignant condition. Indeed, the majority of patients worldwide with HCC have underlying cirrhosis, it is uncommon to find HCC in the absence of cirrhosis [10]. Liver cirrhosis is characterized by destruction of the hepatic lobular architecture and its replacement by nodules containing proliferative hepatocytes, in the presence of chronic inflammation and fibrosis [11].

Another outstanding and important question in HCC pathogenesis involves the role of chromosomal abnormalities that are present almost universally in HCC and frequently detected in hyperplastic and/or dysplastic nodules of cirrhotic livers [12].

Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; TEMs, tetraspanin-enriched microdomains; PI4P, phosphatidylinositol 4-phosphate; PI(4,5)P2, phosphatidylinositol 4,5 bisphosphate; GFP, Green fluorescent protein; TGFβ, transforming growth factor β.

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Some chromosomal defects found in the dysplastic nodules in cirrhotic liver are also present in HCC, which suggests that chromosomal defects occur at early stages of tumor development [13–15]. However, the mechanisms by which chromosomal abnormalities occur are still unknown. Neoplastic evolution of HCC proceeds through a multi-step histological process that is less defined than that of other cancer types. Hyperplastic nodules of regenerating hepatocytes have normal cytological features, and represent a potential first step towards HCC. These lesions can progress to pre-malignant dysplastic nodules, which have abnormal cytological features and these lesions are associated with the increased thickening of the trabeculae, which indicates abnormal liver architecture. The dysplastic nodules can evolve to frank HCC, which is endowed with the capacity to invade the surrounding fibrous stroma and vessels and eventually to metastasize [16,17].

Tumor formation can be switched on from cell membrane domains

It has long been recognized that differences between cancer cells can arise through variation in the extracellular environment or from genomic alteration. It has only recently become clear that plasma membrane protein fluctuations can also have profound effects on the phenotype [18,19]. These fluctuations cause genetically identical cells to vary significantly in their responsiveness to stimuli, for example, those from the fibrotic microenvironment [20–23].

The spatial organization of plasma membrane components in discrete microdomains is thought to be a key factor in the generation of distinct signal inputs or outputs [24]. Dynamic microdomains have important implications for understanding how signaling complexes are assembled and disassembled in response to stimuli; some components of these signaling complexes might reside permanently in these microdomains but others could have extremely transient interactions [25].

This article focuses on the current knowledge regarding the mechanism of neoplastic progression of HCC that can be attributed to the reorganization of the plasma membrane in a functional unit called tetraspanin-enriched microdomains (TEMs). TEMs promote the instability of pre-malignant hepatocytes enhancing their neoplastic progression.

Tetraspanins

Tetraspanins are a large family of proteins (33 in mammals), these proteins have two small and large extracellular loops, four putative hydrophobic membrane-spanning domains and short amino- and carboxy-terminal cytoplasmic domains. Hypervariable region in large extracellular domain of each tetraspanin can distinct it from other members in this family [26]. Tetraspanins (e.g. CD9, CD63, CD81, CD82, CD151) are implicated in numerous cellular pathways including cell proliferation, cell differentiation, cell adhesion, cell fusion, proteins trafficking, migration, viral infections and triggering of immune responses [26,27]. Extracellular domains make tetraspanins being capable to associate with wide range of proteins by homo/heterotypic interactions [28]. It seems these combinations depend on the cell type and differentiation. Treatment with cell membrane detergent following immunoprecipitation and mass spectrometry analysis confirmed that tetraspanins are associated with several kinds of partners [29,30]. The most important tetraspanin partners are integrins, particularly $\alpha3\beta1$, $\alpha4\beta1$, $\alpha6\beta1$ and $\alpha6\beta4$ isoforms, intracellular associated heterotrimeric G proteins, proteases, CD44, EpCAM, immunoglobulin superfamily members and cytosolic signal transduction molecules as well. It is also necessary to mention that different types of tetraspanins in association with various partners produce the variability and specificity among cell types [30] Tetraspanins form complexes termed tetraspanin-enriched membrane microdomains (TEMs) by interacting with other tetraspanins and with a variety of transmembrane and cytosolic proteins that are required for their function [31–33]. Changing the composition and localization of TEMs in response to external or internal stimuli reveal that TEMs are dynamic and flexible structures [34]. TEMs have been characterized by immunoelectron microscopy as units, with an area of $\sim$200 nm^2 although size heterogeneity can be observed in this territory between different cell types [34]. Although the structure of TEMs is similar to that of the lipid rafts, TEMs are evidently distinct from lipid rafts based on the absence of lipid raft-specific glycoproteins such as glycosylphosphatidylinositol-anchored proteins. The interactions of tetraspanin can be stabilized by several modifications including palmitoylation and glycosylation. CD9, CD53, CD81, CD82, CD63, CD151 and CD37 are covalently palmitoylated in Golgi apparatus. Palmitoylation plays an essential role in tetraspanin-protein or tetraspanin-lipid interactions and protect tetraspanins from protease attack [28,35]. Since tetraspanins, as well as most of their partners, are highly palmitoylated membrane proteins, the interactions between proteins and lipids are an important component in the function of TEMs. The characterization of the protein compositions of TEMs has revealed different categories of proteins in these microdomains and the recent advent of the proteomic analysis has allowed the characterization of TEMs in different cancers [30,36]. However, much has to be done to functionally characterize the resulting signals from protein–protein and protein-lipid interactions in TEMs. One of the limitations of the experimental approach is due to the fact that anti-tetraspanin antibodies interfere not only with the targeted tetraspanin but also with the entire microdomain. RNA interference of single or multiple genes involved in TEMs, as well as dynamic studies of microscopy such as fluorescence recovery after photobleaching (FRAP) or Förster resonance energy transfer (FRET), may help to overcome this limitation.

Tetraspanins in HCC

HCC is a disease characterized by heterogeneous morphological patterns. Since HCC arises in a cirrhotic liver, the most relevant aspect of hepatocarcinogenesis is regeneration of hepatocytes. HCC develops more frequently in cirrhosis of the macronodular type than in micronodular cirrhosis, and even more when macroregenerative nodules (MRNs) are present. MRNs, particularly atypical MRNs, develop as a result of extensive regeneration in a cirrhotic liver. The development of HCC in a cirrhotic liver underlines the importance of these early events indicative of neoplastic transformation. In this regard, the tetraspanins CD81 and CD151 are by far the most comprehensively studied (Fig. 1).

Tetraspanin CD81

Tetraspanin CD81 was identified originally as the target of an anti-proliferative antibody (TAPA-1) that inhibited *in vitro* cellular proliferation [37]. Data obtained from monoclonal antibodies have shown that this 26 kDa membrane protein is involved in a broad range of cellular functions. These antibodies evoke their effects by mimicking a natural ligand or by altering the interactions between CD81 and its associated proteins. Although CD81 is widely expressed, its levels of expression within a single tissue vary in response to cellular activation [38]. The ability to associate with itself to form homodimers besides interaction with various other receptors is an important feature of CD81 function [39]. Up-regulation of CD81 in pre-malignant hepatocytes can contribute to