Involvement of ADAMs in tumorigenesis and progression of hepatocellular carcinoma: Is it merely fortuitous or a real pathogenic link?

Antonio Mazzocca *, Gianluigi Giannelli, Salvatore Antonaci

Department of Internal Medicine, Immunology and Infectious Diseases, Section of Internal Medicine, University of Bari Medical School, Piazza G. Cesare 11, I-70124 Bari, Italy

Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and one of the most frequent types of cancer worldwide. It normally develops in patients with chronic liver disease, especially cirrhosis, although some cases without an apparent underlying liver disease have been reported. The pathogenesis of HCC is multi-factorial and complex. Hepatitis viruses are the main factors favoring the development of HCC. In fact, chronic inflammation associated with hepatitis C or B virus infection can lead to progressive liver fibrosis, cirrhosis and ultimately HCC. Chronic inflammation and liver fibrosis cause a continuous remodeling of the extracellular matrix (ECM), a dynamic process that involves several molecules including integrins and matrix processing enzymes. An increasing body of evidence indicates that ADAMs are involved in promoting tumor formation and progression of HCC. A Disintegrin And Metalloproteases (ADAMs) are a group of proteins belonging to the zinc protease superfamily. ADAMs are usually transmembrane proteins that contain disintegrin and metalloprotease domains and are, therefore, able to carry out both cell adhesion and protease activities. Soluble isoforms of ADAMs have also been discovered and characterized. In this review, we focus on the contribution of ADAM proteins to HCC tumorigenesis and cancer progression. The potential role of ADAMs as key modulators of tumor–stroma interactions during tumor progression, by means of the activities of their constituent domains, is also discussed.

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The ADAM family proteins are a group of transmembrane metalloproteases that belongs to the superfamily of zinc-proteases. The members of this family of proteins share the following structural domains: a pro-domain, a metalloproteinase domain, a disintegrin-like domain, a cysteine-rich domain, an EGF-like domain, a transmembrane and a short intracellular domain. These domains are essential for the catalytic activity of ADAMs, allowing them to cleave various substrates.

2. The ADAMs: a class of multidomain and multifunctional proteins

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3. ADAMs with a proven role in cancer

The relationship between ECM and the infiltrating tumor tissue is largely regulated by enzymes able to degrade the ECM, which are mostly produced by stromal cells. Several studies carried out over the last decade have established that many families of enzymes degrading the ECM are involved in tumor progression and the metastatic process [37–39]. Historically speaking, the earliest characterized family of enzymes degrading the ECM is that of conventional matrix metalloproteases (MMPs) [40]. Proteolytic modification of ECM profoundly alters the microenvironment and contributes to different steps in tumor formation and progression. Recently, ADAM proteins have received close attention because they may reveal important regulators of the crosstalk between different cell types.

![Fig. 1. Structure of ADAMs. A. Conserved domains of ADAM transmembrane proteins include the signal sequence (SS), pro-domain, metalloproteinase domain (includes a zinc (Zn) binding site), and disintegrin and cysteine-rich domains. ADAMs also contain EGF-like and transmembrane domains (TM) and a cytoplasmic (Cyt) tail. B. Soluble isoforms generally lack part of the juxta-membrane domain at the cysteine-rich or EGF-like level and hence the anchorage to the plasma membrane.](image-url)
cancer cells and the tumor microenvironment. Several studies, recently reviewed by Duffy MJ et al. [10], have shown a fortuitous role of ADAMs in cancer (Table 1). Some of the first evidence of the involvement of these molecules in cancer came from an analysis showing differences in the expression levels of ADAMs members, including ADAM9, ADAM10, ADAM12 and Metarginin (ADAM15) in various hematological malignancies [41]. Later, it was shown that ADAM15 is associated with the integrin receptor αvβ3 in melanoma cells, suggesting a potential role in integrin-mediated neo-vascularization and cancer progression [42,43]. More recently, using a mouse model of prostate cancer, Najy et al. have demonstrated that ADAM15 modulates the metastatic capability of tumor cells to colonize the bone, likely affecting the interaction between the endothelium and cancer cells [44]. Different studies have shown that ADAM12 is highly expressed in many types of cancer and tumor cells and that ADAM12 may regulate important cellular functions (i.e. between cells, or cells and the ECM) through its interaction with adhesion molecules such as integrins and syndecans [45]. For example, ADAM12 preferentially binds to α9β1 but in cases where this receptor is not expressed, as in many carcinomas, it could bind to other members of the β1 integrin family [46]. Also, ADAM12 interacts with syndecan-4 and this interaction can promote cell spreading and stress fiber assembly [47]. A functional study by Kvěibor et al. [48] demonstrated that ADAM12 accelerates tumor progression in a murine breast cancer model. In addition, they found indications that ADAM12 regulates tumor–stroma interactions by affecting the apoptosis of stromal cells. The involvement of ADAM9 in cancer has also been investigated. Over the last decade, studies conducted by different research teams have attempted to shed light on the role of this ADAM in cancer progression. For instance, it has been reported that in breast carcinoma, increased expression levels of ADAM9 are correlated with cancer progression [29] and that ADAM9 is highly expressed in renal cancer, where it is significantly correlated with markers of poor prognosis [49]. In prostate cancer ADAM9 is over-expressed and seems to be an independent prognostic marker of disease-free survival following radical prostatectomy [50]. In addition, the expression of ADAM9 in prostate cancer can be regulated by intracellular reactive oxygen species and/or hydrogen peroxide generated when the cells are exposed to stress conditions such as cell crowding and hypoxia [51]. In an interesting study by Peduto et al. [52], it was found that transgenic overexpression of ADAM9 in mouse prostate results in an abnormal glandular epithelium and high-grade prostatic intraepithelial neoplasia (PIN), whereas mice lacking ADAM9 form well-differentiated tumors; thus this study unveiled a key role of this molecule in prostate carcinogenesis. They also suggested that the shedding of EGF and FGFR2 operated by ADAM9 can be a potential mechanism underlying prostate carcinogenesis. Another study, using a laser microdissection and pressure catapulting technique, showed that ADAM9, along with ADAM15, is

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<th>Adam</th>
<th>Expression in cancers</th>
<th>Function in tumorigenesis</th>
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<tr>
<td>ADAM8</td>
<td>Up-regulated in most cancers including renal cell carcinomas and astrocytomas</td>
<td>Tumor cell invasion</td>
<td>[Rev. in 8]</td>
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<tr>
<td>ADAM9</td>
<td>Liver, renal, prostate, pancreas, gastric, lung, and breast</td>
<td>Bending to integrins (α6β1 and α2β1); tumor cell adhesion, invasion and regulation of tumor–stroma interactions; expression induced by hypoxia and oxidative stress; high-grade PINa in mouse overexpressing ADAM9; brain metastasis in a mouse model of NSCLCb</td>
<td>[49–58]</td>
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<td>ADAM10</td>
<td>Prostate, breast, ovary, uterus and hematological malignancies</td>
<td>Shedding of Notch, CD44, E-cadherin and EGFR ligands</td>
<td>[Rev. in 10,11,41]</td>
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<td>ADAM12</td>
<td>Liver, colon, gastric, breast, and hematological malignancies</td>
<td>Binding to integrins (α9β1); interaction with syndecan-4; regulation of tumor–stroma interactions; up-regulated in HCCc</td>
<td>[32,41.45–48,56]</td>
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<td>ADAM15</td>
<td>Prostate, melanoma, pancreatic, gastric, and hematological malignancies</td>
<td>Binding to integrins (αvβ3); neo-vascularization in --/--; bone metastasis in a mouse model of prostate cancer</td>
<td>[41–44,53,56]</td>
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<td>ADAM17</td>
<td>Renal, liver, colon, gastric, breast, brain, and ovary</td>
<td>Shedding of TGF-α and EGFR activation in renal carcinoma; knockdown suppresses formation of renal carcinoma in nude mice; up-regulated in poorly differentiated HCC; cleavage of membrane-bound amphiregulin precursor in the early phases of hepatocarcinogenesis; target of microRNA (miR-122) a tumor suppressor that reduces HCC progression in an animal model</td>
<td>[59,82,90,91]</td>
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<td>ADAM19</td>
<td>Overexpressed in brain tumors and renal cell carcinomas</td>
<td>No functional study</td>
<td>[Rev. in 8]</td>
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<td>ADAM28</td>
<td>Breast and lung</td>
<td>Up-regulated in human breast carcinomas; knockdown inhibits breast cancer growth in a xenograft model; correlation with mitotic activity in human breast cancer and NSCLC</td>
<td>[60,61]</td>
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a PIN = prostatic intraepithelial neoplasia.
b NSCLC = non-small cell lung cancer.
c HCC = hepatocellular carcinoma.
highly expressed in pancreatic cancer as compared to normal pancreatic tissues [53]. A similar study, done by gene profiling, revealed that ADAM9 is up-regulated in different pancreatic cell lines [54]. In addition, Grutzmann et al. [55] demonstrated that the expression of ADAM9 is associated with certain types of pancreatic cancer and can be considered a reliable prognostic factor in ductal adenocarcinoma. Evidence of significantly up-regulated ADAMs, including ADAM9, ADAM12 and ADAM15, was also provided in an analysis of gastric cancer [56]. Moreover, overexpression of ADAM9 was found to be correlated with the capacity of non-small cell lung cancer (NSCLC) cells to seed metastasis to the brain [57]. Interestingly, ADAM9 was one of the genes de-regulated by recurrent high-level amplifications associated with reduced survival duration in patients with breast cancer [58]. Besides ADAM9, ADAM12 and ADAM15, other ADAMs have been implicated in cancer formation and/or progression. For example, ADAM17 regulates important autocrine signaling pathways through its sheddase activity on TGF-α that is essential for an efficient EGFR activation in renal carcinoma cells. Moreover, when ADAM17 is knocked down by RNA interference this is sufficient to suppress renal carcinoma formation in nude mice, suggesting that pharmacological targeting of ADAM17 could be beneficial in this type of cancer [59]. ADAM28 and its soluble isoform, ADAM28-S, are up-regulated in human breast carcinomas as compared to non-neoplastic tissues and are correlated with the mitotic activity of carcinoma cells. In a xenograft model, silencing of ADAM28 inhibits the growth of breast cancer cells [60]. In addition, the same study showed that ADAM28 cleaves IGF8β-3, making unbound IGF-I more available to act on its receptor. In another study, the same authors demonstrated that ADAM28 is overexpressed and activated in human non-small cell lung carcinomas as compared to non-carcinoma lung tissues, and that this is correlated with a higher mitotic cell index [61].

4. Tumor–stromal interactions during HCC progression

The crosstalk between stroma and tumor cells is unquestionably relevant to tumor progression. In particular, during the transition from carcinoma in situ to invasive carcinoma, the cancer cells penetrate through the basement membrane and infiltrate the surrounding stroma. Pathologists consider this a clear sign of malignancy. As a consequence of this infiltration, tumor cells gain access to lymphatic and/or blood vessels and eventually to the systemic circulation. Important changes in the stroma distribution, organization and composition occur during the acquisition of the invasive phenotype [62,63]. In HCC, the stromal component is very different (structurally and qualitatively) from that of normal liver tissue but seems to more closely resemble that of cirrhotic liver, of which HCC often originates [64]. HCC stroma is made up of several cell types, including immune cells, inflammatory cells, smooth muscle cells, fibroblasts and myofibroblasts, activated Ito cells, Kupffer and endothelial cells [65]. The stroma of HCC is also composed of ECM components and soluble factors (i.e. growth factors and cytokines) that play an active role during tumor progression [66–68]. Using various experimental models it has been demonstrated that the tumor microenvironment determines the efficiency of neoplastic transformation, growth rate, degree of invasiveness, and the ability to form distant metastases in HCC [69,70]. Like in most carcinomas, the influence of the microenvironment in HCC is in large part mediated by paracrine signals between cancer cells and stromal cells (fibroblasts/myofibroblasts, endothelial cells and other cell types) [71,72]. Myofibroblasts are generally the main cellular element determining the reactive stroma in HCC [65]. Myofibroblasts also play an important role in epithelial–stromal interactions in the tumor microenvironment at the invasive front. Furthermore, myofibroblasts are detectable in a higher proportion

Fig. 2. ADAMs promote tumorigenesis through different mechanisms including the regulation of tumor–stromal interactions. Schematic model illustrating the mechanism by which ADAMs interact in the crosstalk between tumor and stromal cells. Tumor cells can recruit stromal cells by secreting growth factors (GFs) and cytokines, which in turn act on tumor cells by producing soluble factors, ECM and a variety of enzymes including ADAMs. Transmembrane ADAMs shed many cell-surface proteins including growth factors, which in turn become activated and exert their biological activity in an autocrine or paracrine fashion. This activity can be exerted by growth factors and cytokines on both tumor and stromal cells, thereby stimulating tumor growth and/or stromagenesis. For example, ADAM17 can be expressed by tumor or stromal cells and shed heparin-binding EGF-like growth factor (HB-EGF). This growth factor acts on both tumor and stromal cells, thus promoting tumor growth. Moreover, ADA17 is overexpressed in most poorly differentiated HCC and it can be inhibited by miR-122, which exerts a tumor suppressor action. In the case of soluble ADAM9 (ADAM9-S), this is released by stromal cells and binds to integrins (α6β4 and α2β1) on the surface of tumor cells. Through its proteolytic activity, ADAM9-S then promotes invasion by degrading components of the ECM, thus allowing tumor cells to invade the surrounding tissues.
of invasive cancers as compared to carcinomas in situ [73]. Myofibroblasts are able to produce enzymes that degrade the ECM, a prerequisite for tumor invasion. The cancer-associated myofibroblasts are largely responsible for the so-called “desmoplastic reaction” as they are able to synthesize and secrete large quantities of collagen and other ECM components. They are also able to contract, on account of their smooth muscle actin and myosin. The abundant deposition of collagen and tissue contraction are responsible for the dense, hard consistency of tumors [74]. Myofibroblast differentiation is initially induced by cytokines released by tumor cells from carcinoma in situ [75,76]. There are also many indications that tumor-associated myofibroblasts acquire an increased ability to support tumor growth as compared with myofibroblasts from the normal surrounding tissue [77]. They are also believed to provide a favorable environment for the seeding and development of metastases [78], so targeting tumor–stroma is a new, emerging anti-cancer strategy [79,80]. This strategy is aimed at interrupting the molecular crosstalk between tumor and stromal cells that supports invasive and metastatic growth, through the inhibition of those factors that play a key role in mediating tumor–stroma interactions. Stromal cells are abundant in metastases, suggesting that the metastatic cell continues to be dependent on stromal cells for optimal autologous outgrowth in the invaded organ. The characteristics of the specific tissue microenvironment likely play a key role in inducing susceptibility to metastatic transformation.

5. The contribution of ADAMs to HCC oncogenesis and tumor progression

There is an increasing body of evidence that ADAM proteins play an active role in the pathogenesis of HCC. In Fig. 2, a schematic model summarizes some of the mechanisms in which ADAMs participate during hepatocarcinogenesis. In an effort to identify novel HCC-associated proteins using protein microarrays, Tannapfel et al. [81] found that ADAM9 is up-regulated in HCC as compared to non-neoplastic liver tissue. Similarly, the expression levels of ADAM17 mRNA were significantly higher in poorly differentiated than in moderately or well-differentiated HCC, and this was correlated with HCC progression [82]. At the same time, Le Pabic et al. [32] showed that the mRNA levels of ADAM9 and ADAM12 are significantly increased in HCC patients as compared to normal subjects or those with benign tumors, and that ADAM12, but not ADAM9, is under the transcriptional control of TGF-β1 in Ito cells. TGF-β1 is an important and controversial regulator in cancer. In normal and premalignant conditions, it seems to act as a tumor suppressor with a bivalent action on both tumor and stromal cells whereas it acts as a tumor promoter in advanced phases of tumor progression [83]. For example, it has been shown that TGF-β1 activates the EGFR-mediated survival signaling pathway in fetal rat hepatocytes through the involvement of TACE/ADAM-17, and that TGF-β1 suppresses adult quiescent hepatocytes but not hepatoma cells that escape from this suppressor effect, thereby acquiring a more aggressive phenotype [84,85].

In another study, Mazzocca et al. [86] showed that a soluble form of ADAM9 (ADAM9-S) secreted by activated myofibroblasts/hepatic stellate cells promoted tumor invasion by means of stromal–tumor interactions in different carcinoma cell lines. ADAM9-S, secreted by stromal cells, acts on tumor cells in a paracrine fashion through direct binding to α6β4 and α2β1 integrins. In this way, the tumor cell surface can be “armed” with a fresh proteolytic activity that is able to degrade the surrounding matrix [86]. It is important to point out that in our analysis of liver tissues the expression of ADAM9 was rarely detected in carcinoma cells and never observed in normal hepatocytes (data not shown). On the contrary, it was mostly expressed by α-SMA-positive/activated myofibroblasts in both HCC and cirrhotic liver tissues, suggesting the idea that the expression of this protease is related to the activation state of this important component of the hepatic stroma. In the same way, ADAM9 expression in liver metastatic colon carcinoma is prevalently detected in the stromal cells within the tumor corresponding to activated liver myofibroblast/α-SMA-positive cells (Fig. 3). A study by Schwettmann et al. [19] has recently shown a strong correlation between ADAM9, ADAM28 and ADAMTS1 with MMP-2 and MMP-9.
The development of appropriate animal models and more detailed the role played by these molecules in liver diseases, particularly in HCC. Since chronic liver diseases with sustained fibrosis represent an important risk factor for developing HCC, it is reasonable to assume a contribution of these molecules during the initial phases of hepatocarcinogenesis. Recent studies have demonstrated that ADAMs control important oncogenic signaling pathways in HCC cells. The molecular crosstalk between angiotensin II and EGFR leads to carcinoma. Recent studies have demonstrated that ADAMs have suggested that the cleavage of the membrane-bound AmphiRin precursor by ADAM17 is a mechanism that takes part in the early stages of hepatocarcinogenesis. In agreement with this, is the observation that ADAM17 is up-regulated in both cirrhotic liver, a precancerous condition, and in HCC tissues but not in healthy controls [90]. Lastly, a tumor suppressor microRNA called miR-122 has been shown to reduce intraperitoneal HCC metastasis by inhibiting angiogenesis. One of the target genes of this tumor suppressor microRNA is ADAM17, whose inhibition by RNA interference results in a reduced tumor progression in a nude mouse model [91].

6. Concluding remarks

This review has examined the involvement of ADAM proteins in tumorigenesis and cancer progression, focusing particularly on HCC. In this type of cancer, different studies have demonstrated a significant link between some members of the ADAM proteins superfamily and the pathogenic mechanisms underlying HCC formation. ADAM proteins are involved in various regulatory mechanisms during HCC progression. These molecules act as regulators of tumor cell functions and the relationship between tumor and stromal cells. Such mechanisms can be autocrine or paracrine and regulate cell proliferation, cell movement and invasion of tumor cells. Autocrine and paracrine mechanisms can occur through the shedding of growth factors and cytokines, which in turn results in the activation of their receptors and signaling pathways, or through the action of soluble isoforms of certain ADAMs. Alternatively, ADAMs can bind to integrins or other adhesion molecules via their disintegrin and cysteine-rich domain and, in this way, ADAMs can modulate the function of these molecules. Despite these valuable findings on ADAMs in cancer, we are at the dawn of understanding of the role played by these molecules in liver diseases, particularly in HCC.

The development of appropriate animal models and more detailed large-scale clinical analyses may contribute to a better knowledge of the role of ADAMs, and hopefully lead to more and more translational applications of the obtained results in patients with liver cancer.

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