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TOPIC HIGHLIGHT

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Activins and activin antagonists in hepatocellular carcinoma

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Abstract

In many parts of the world hepatocellular carcinoma (HCC) is among the leading causes of cancer-related mortality but the underlying molecular pathology is still insufficiently understood. There is increasing evidence that activins, which are members of the transforming growth factor β (TGFβ) superfamily of growth and differentiation factors, could play important roles in liver carcinogenesis. Activins are disulphide-linked homo- or heterodimers formed from four different β subunits termed βA, βB, βC, and βE, respectively. Activin A, the dimer of two βA subunits, is critically involved in the regulation of cell growth, apoptosis, and tissue architecture in the liver, while the hepatic function of other activins is largely unexplored so far. Negative regulators of activin signals include antagonists in the extracellular space like the binding proteins follistatin and FLRG, and at the cell membrane antagonistic co-receptors like Cripto or BAMBI. Additionally, in the intracellular space inhibitory Smads can modulate and control activin activity. Accumulating data suggest that deregulation of activin signals contributes to pathologic conditions such as chronic inflammation, fibrosis and development of cancer. The current article reviews the alterations in components of the activin signaling pathway that have been observed in HCC and discusses their potential significance for liver tumorigenesis.

Key words: Hepatocellular carcinoma; Activin; Follistatin; Transforming growth factor β

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the predominant form of primary malignancy of the liver and accounts for more than half a million deaths per year[1]. In some geographical regions it is the most prevalent form of malignancy and the most common cause of death from cancer[2] making its containment a top priority. Chronic infection with the hepatitis B or C virus (HBV, HCV), dietary exposure to the hepatocarcinogen aflatoxin B1 (AFB1), ethanol abuse, and obesity are among the main risk factors for liver cancer[3]. Despite recent advances, the molecular pathology of the disease is not well understood and the therapeutic possibilities are largely limited to surgical procedures including resection, liver transplantation, or local tumor ablation[4]. A consistent pattern of changes comparable to the sequential mutations in tumor suppressor genes and oncogenes, like the one identified in colon carcinogenesis during adenoma to carcinoma progression[5,6], has not been defined for HCC. Nevertheless, multiple genetic alterations including mutations of p53, inactivation of the Rb pathway, and activation of the Wnt/β-catenin pathway have been linked to HCC development and progression[3,7]. In addition deregulated expression of growth factors and their cognate receptors has been described in HCC for both, positive regulators of hepatocyte growth, such as insulin-like growth factor 2 (IGF-2), hepatocyte growth factor (HGF), and transforming growth factor α (TGFα), as well as for negative regulators like TGFβ[8].

In recent years activins, a subgroup of the TGFβ family of growth, differentiation, and death factors which share part of their signaling mechanisms with TGFβ, have gained attention with respect to their role in tumor development in several organs[9]. Activin subunits, their receptors and several antagonistic proteins are expressed in the normal liver. Deregulation of this balanced expression

appears to contribute to hepatic dysfunctions like impaired regeneration, fibrogenesis and tumorigenesis[10]. The current knowledge about the role of activins and activin antagonists in HCC is discussed in this review.

ACTIVINS-STRUCTURE AND SIGNALING

Activins are secreted polypeptides and represent a subgroup of the TGFβ superfamily of growth and differentiation factors. Additional members of this superfamily include TGFβ1-3, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), myostatin, Muellerian inhibiting substance (MIS), nodal and several others[10,11]. Activins are homo- or heterodimers composed of four different β subunits (βA, βB, βC, βE), each encoded by a single gene. The β subunits can either form activins by dimerization with a second β subunit, or alternatively can form inhibins by dimerizing with a single α subunit encoded in mammalian genomes[12]. Activin terminology is dependent on the dimer configuration with a single letter designating homodimers (activins A, B, C, and E) and two letters designating heterodimers according to their subunit composition (activins AB, AC, AE, BC *etc.*). With respect to tissue expression, transcripts of the βA and βB subunits were found to be detectable in almost all tissues analyzed with especially high expression in reproductive organs[13,14]. The βC and βE subunits, in contrast, are predominantly expressed in the liver and at lower levels in a limited number of additional organs[14-18].

Activin β subunits are synthesized as precursor molecules with 350-426 amino acids and molecular weights between 38 kDa and 50 kDa[19]. The prodomains are removed in the ER and in the early Golgi by members of the protease family of subtilase-like pro-protein convertases (SPC)[20] to release mature peptides with either 115 (βB, βE) or 116 (βA, βC) amino acids. The amino acid sequences of the mature peptides are approximately 50% conserved among the four human β subunits, whereas the sequence homology in the prodomain is only about 20%. Analysis of the phylogenetic relationship of the mature human peptides groups together βA and βB on the one and βC and βE on the other hand[21].

Like other members of the TGFβ family, the activin β subunits contain nine conserved cysteines in the mature peptides. The sixth is used for dimerization, whereas the other eight form intramolecular disulfide bonds which determine the three-dimensional structure of the peptides[22]. While all cysteines in the mature chain of activin βA are necessary for biosynthesis of activin A dimers or for their full biological activity, four additional cysteines in the prodomain are dispensable for dimerization and secretion. Protein folding and dimerization take place in the lumen of the ER and are catalyzed by members of the protein disulfide isomerase (PDI) family[23,24]. Unlike TGFβ, which is secreted as a latent complex consisting of the TGFβ homodimer, its prodomain (also termed latency-associated propeptide, LAP), and the latent TGFβ binding protein (LTBP)[25], activins are secreted as dimers of the mature peptides and need no further processing in the extracellular space to gain bioactivity. Activin A signals are transduced *via* two types of single-pass transmembrane serine threonine kinase receptors, termed activin receptors type I and type Ⅱ[26]. Activin A first binds to the type Ⅱ receptors which in turn recruit and phosphorylate the type I receptors[27]. Two type Ⅱ receptors for activin A (ActR-ⅡA or ACVR2 (A) and ActR-ⅡB or ACVR2B) have been identified. The main type I receptor for activin A is ALK (Activin Receptor-Like kinase) 4, also designated as ActR-IB or ACVR1B, whereas activins B and AB have a preference for ALK 7 (ACVR1C) as type I receptor[28]. Receptors for activins containing βC or βE subunits have not been identified so far. Activin C, however, did not compete with activin A for receptor binding[29] and a chimeric activin construct in which the receptor binding sequence (amino acids 46-78) of βA was replaced by the corresponding region of βC retained type Ⅱ receptor binding but was unable to recruit the type I receptor ALK 4[30].

Inhibins have been shown to form a complex with type Ⅱ receptors via their β subunits and with betaglycan also known as TGFβ type Ⅲ receptor. The α subunit, however, is unable to bind type I receptors and consequently activin receptor signaling is inhibited[31,32]. There is in general a considerable degree of promiscuity in receptor usage by different TGFβ superfamily members. In addition to activin A, for instance, myostatin, and several BMPs were shown to signal *via* ActR-ⅡB[33].

Phosphorylated TGFβ family receptors recruit intracellular mediators called Smads, which transduce activin signals to the nucleus[26]. Smads can be divided into receptor Smads (Smads 1, 2, 3, 5 and 8), a common mediator Smad (Smad 4) and inhibitory Smads (Smads 6 and 7). Activin A receptors, as well as TGFβ receptors, recruit and phosphorylate the receptor Smads 2 and 3, whereas receptor Smads 1, 5, and 8 are recruited by BMP receptors but not activin receptors[34]. Recent evidence suggests that-similar to TGFβ-additional Smad-independent signaling pathways may contribute to activin A signaling, as for instance, RhoA, MEKK1, JNK, and p38 were found to be involved in activin-induced cytoskeleton reorganization and cell migration in keratinocytes and in promoter activation of the transcription factor Pit-1 in pituitary lactotrope cells[35,36].

Activin signals are tightly regulated on the one hand by a spatially and temporally restricted production of activin subunits and on the other hand by the expression of several extra- as well as intracellular antagonists of activin signaling. An overview of activin-mediated signaling events and the corresponding interaction points with endogenous activin antagonists is presented in Figure 1.

ACTIVIN SUBUNITS AND ACTIVIN ANTAGONISTS IN LIVER CANCER

Activin βA

Activin A, the homodimer of two βA subunits, is by far the most extensively investigated activin. Multiple biological functions of activin A in a variety of cells and tissues have been described. Activin A has been implicated for instance in mesoderm induction[37], stem cell biology[38], reproductive biology[39], erythroid differentiation[40], systemic inflammation[41], cell death induction[42], wound healing[43],

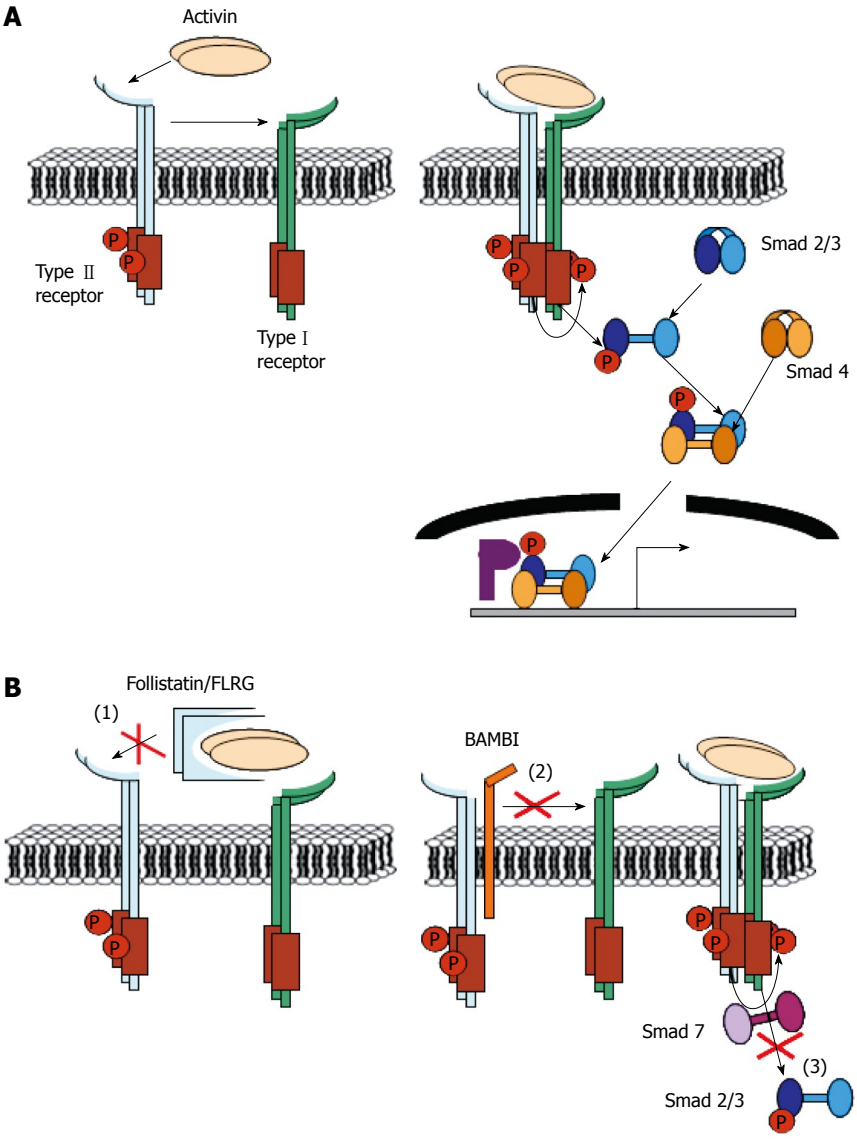

Figure 1 Graphic representation of activin signaling and interaction points with activin antagonists. **A**: Activin dimers first bind the type II activin receptors, which then recruit and phosphorylate type I receptors. These in turn phosphorylate receptor-activated Smads, which subsequently form a complex with Smad 4 and are translocated to the nucleus, where they regulate the transcription of target genes; **B**: Activin antagonists can block activin signals by: (1) Binding activins in the extracellular space like follistatin or FLRG and thereby blocking their access to activin receptors; (2) Acting as inhibitory co-receptors, which prevent ligand receptor interactions (Cripto) or receptor dimerization (BAMBI); (3) Competing with receptor-activated Smads 2 and 3 for binding sites on activin receptors (Smad 7).

and fibrosis[44]. Knock-out mice for βA have severe defects in craniofacial development and die shortly after birth[45]. Concerning the liver, activin A potently inhibits mitogen-induced DNA synthesis and induces apoptosis in hepatocytes *in vivo* and *in vitro*[46-48]. Activin βA antisense oligonucleotides stimulated cell proliferation in the human hepatoma cell line HLF suggesting a growth inhibitory function of endogenous activin A[49]. In regenerating liver, activin βA gene expression was reduced at time points when hepatocyte replication took place and was increased at later periods when liver regeneration terminated[50]. Increased expression of βA at earlier time points after partial hepatectomy, however, has also been described[51,52]. Besides the effects on DNA synthesis and cell growth, activin A also regulates restoration of liver architecture after partial hepatectomy by stimulating collagen production in hepatic stellate cells (HSC) and tubulogenesis of sinusoidal endothelial cells[53,54]. Stimulation of HSC may also contribute to liver fibrosis and several investigations have found elevated levels of activin βA in fibrotic and cirrhotic rat livers[55-58]. Elevated levels of circulating activin A were found in patients suffering from chronic viral hepatitis or alcohol induced liver cirrhosis and in HCC patients[59-61]. Reduced expression of activin βA transcripts in contrast, was observed in tumor tissue from chemically-induced rat liver tumors and in 5 of 11 HCC specimens[62]. In addition to a pro-apoptotic effect on the parenchymal cells and a pro-fibrotic effect on HSC, activin A has also been linked to neoangiogenesis *via* stimulation of VEGF expression in human hepatoma cells[63].

Activin βB

Like activin βA, the βB subunit is expressed in multiple tissues and organs[13,14]. Despite a considerable overlap in tissue expression and in some biological activities, important differences exist[64]. Knock-out mice for βB are viable but have defects in eyelid development and female reproduction[65]. When the coding region of the mature peptide of the βA subunit gene was replaced with the corresponding region of the βB subunit gene the developmental defects of the βA knock-out mice were only partially rescued[66]. Concerning the liver, the role of the βB subunit is not well characterized. In the normal rat liver the βB subunit was the only activin subunit undetectable by RNAse protection assay[14]. Weak positive immunoreactivity for βB was, however, detected in hepatocytes of normal

rat livers and in connective tissue septa in fibrotic livers when analyzed by immunohistochemistry[55]. Activin βB mRNA was induced in stellate cells of CCl_4 treated rat livers[55]. Exposure to the peroxisome proliferator di-n-butyl phthalate led to a transient surge of βB mRNA expression 6 h after treatment in rat livers[67]. With respect to biological activities, recombinant activins A and AB but not activin B inhibited EGF induced DNA synthesis in primary rat hepatocytes[68]. In normal human liver the βB transcript is readily detectable by RT-PCR (M.G. unpublished observation), but no data with regard to expression changes of the βB subunit in liver tumors compared to normal liver have been reported yet.

Activin βC

The activin βC subunit was cloned from liver cDNA and demonstrated to be predominantly expressed in hepatocytes by Northern blot analysis and RNAse protection assays[14,18,52,69]. By immunohistochemistry significant activin βC expression has been detected in cells from additional organs including the prostate, ovary, testes, and pituitary gland[15,70]. Formation of homodimeric activin C as well as heterodimeric activins AC, BC, CE, as well as inhibin C has been demonstrated by ectopic expression of the respective subunits in different cell models[14,70,71]. After partial hepatectomy a transient down-regulation of activin βC expression was observed by several studies[50,52,72,73]. A decrease of activin βC expression has also been observed in HepG2 and Hep3B hepatoma cells versus normal liver tissue[74] and in rat hepatocytes during primary culture with and without EGF treatment[52]. In contrast, increased activin βC expression was reported in the rat liver during the development of CCl_4 induced cirrhosis[56,75] and in response to treatment with the peroxisome proliferator bi-n-butyl phthalate[67]. The functions of the activin βC subunit are controversial. Activin βC knock-out mice developed normally and liver regeneration after partial hepatectomy proceeded similarly in knock-out animals and wild-type littermates[76]. Ectopic expression of activin βC induced apoptosis in human (HepG2, Hep3B) and rat (H4ⅡEC3) hepatoma cells and delayed liver regeneration in mice[74,77]. In AML12 cells, an immortalized mouse hepatocyte cell line in contrast, and in primary rat hepatocytes, activin βC increased DNA synthesis[29]. Adenovirus-mediated expression of activin βC accelerated liver regeneration after partial hepatectomy in rats[78]. A specific association of activin βC immunoreactivity with mitotic hepatocytes was observed in regenerating liver after partial hepatectomy[50]. It was shown that activin C does not activate activin A-responsive promoters, and it was suggested that the βC subunit regulates the levels of bioactive activin A *via* the formation of signaling-incompetent activin AC heterodimers in PC3 human prostate cancer cells[79,80]. Data regarding the expression of the βC subunit in human liver tumors are not available yet.

Activin βE

Similar to activin βC, the βE subunit is predominantly expressed in hepatocytes but has also been detected in human heart, testis, peripheral blood leucocytes, placenta, and skeletal muscle[14,16,21,81]. Formation of homodimeric activin E as well as heterodimeric activins AE and CE has been demonstrated after ectopic co-expression of the respective subunits[14,16]. Activin βE mRNA expression was transiently up-regulated after partial hepatectomy or portal vein branch ligation[73,76] and in response to lipopolysaccharide treatment[81]. Increased βE expression has also been observed in hepatic fibrosis induced by CCl_4[75]. Recently, induction of βE expression has been described as a marker for phospholipidosis in HepG2 hepatoma cells[82]. Similar to βC, βE subunit knock-out mice and double knock-outs lacking both βC and βE expression developed normally and had no defects in liver function[76]. When ectopically expressed in HepG2 or Hep3B hepatoma cells or in the murine hepatocyte cell line AML12, activin βE reduced cell number and increased apoptosis rates[74,83]. Transient overexpression of βE by non-viral gene transfer in the mouse liver inhibited regenerative DNA synthesis[77]. These observations suggest that activin E may have a growth-limiting function similar to activin A, however, the two subunits show a reciprocal pattern with respect to diurnal variations of expression[10]. In line with a growth-limiting function of activin E, transgenic mice overexpressing βE in the pancreas showed reduced proliferation of pancreatic exocrine cells[84]. Regarding liver cancer, reduced expression of the βE subunit was found in human HCC specimens as well as in N-nitroso morpholine-induced rat liver tumors[62,85]. Interestingly, activin βE expression was found to be regulated by the tumor suppressor gene RASSF1A[86], a gene frequently inactivated by promoter hypermethylation in HCC[87,88].

Inhibin α

The inhibin α subunit is part of inhibins but not activins and in many biological systems activins and inhibins have antagonistic effects[89]. Historically activins received their name from the fact that they activated follicle stimulating hormone (FSH) secretion from the pituitary, whereas the previously described inhibins represented the long sought-after gonadal feed-back inhibitor of pituitary FSH secretion[12]. Knock-out mice for the inhibin α subunit developed gonadal sex-cord stromal tumors suggesting a tumor suppressive function of the inhibin α subunit[90]. In several human tumor types including some types of ovarian carcinoma and adrenal tumors, in contrast, overexpression of inhibin α has been demonstrated, and inhibins have been used as serum markers for early detection of ovarian germ cell tumors and monitoring of recurrence[9]. With regard to liver cell growth, treatment with inhibin A *per se* had no effect on DNA synthesis of HepG2 hepatoma cells but antagonized the inhibitory effect of activin A[91]. In normal and fibrotic rat liver absence of inhibin α subunit immunoreactivity has been reported[55]. Immunostaining for inhibin α has been used to distinguish adrenal cortical tumors, which are positive in about 70% of cases, from HCC and renal cell carcinoma, which are mostly negative[92,93].

Follistatin

Follistatin is a secreted, monomeric glycoprotein lacking homology to the TGFβ superfamily. The biological

activities described for follistatin, however, seem to depend entirely on its interaction with activins and other members of the TGFβ family. Follistatin is expressed in most of the organs, that also express activin[13,94], and it binds mature secreted activin A with very high affinity (Kd 50-680 pmol/L)[95-97]. Complex formation with follistatin completely abolished receptor binding of activin A, thus blocking activin signaling[96,98]. Two follistatin molecules embrace one activin dimer and bury one-third of its residues and its receptor binding sites[99]. Three major forms of secreted follistatin exist, resulting from alternative splicing and protein processing of a single follistatin gene and containing 288, 303 and 315 amino acids, respectively[95]. All forms of follistatin contain three homologous follistatin domains[100] of which the first two, but not the third, are necessary for activin A binding[97,101]. Follistatin 288 binds to heparan sulfates, whereas this binding is blocked by an acidic tail in follistatin 315[95]. In addition to binding activins A, B, AB, and E, follistatin was also shown to bind and antagonize myostatin as well as BMPs 2, 4, 6 and 7[16,102-105]. Follistatin administration by intraportal infusion or adenovirus-mediated overexpression caused DNA synthesis and liver growth in normal rat livers presumably by antagonizing tonic inhibition of liver growth by activin A[106,107]. Following partial hepatectomy follistatin expression was up-regulated after 24-48 h, the time period in which hepatocyte replication was increased[50]. Under similar conditions administration of follistatin accelerated liver regeneration but led to impaired restoration of normal tissue architecture and compromised liver function[108-110]. Administration of exogenous follistatin in CCl_4 treated rats attenuated the formation of liver fibrosis[111]. These results likely reflect the ability of follistatin to antagonize both growth-inhibitory and pro-fibrotic activities of activin A.

In human liver cancer and also in animal models follistatin expression was increased in about 60% of tumor tissues. Increased follistatin levels were also found in the blood of patients with liver cirrhosis and HCC[60,62,112]. Administration of follistatin stimulated DNA synthesis in preneoplastic rat hepatocytes in an *ex vivo* system, whereas hepatoma cell lines were unresponsive to exogenous follistatin possibly due to autocrine production of follistatin or other activin antagonists[62,112-114].

FLRG

Follistatin-related protein, encoded by follistatin-related gene (FLRG), also designated as follistatin-like 3 (FSTL-3) has a high similarity to follistatin and shares its ability to bind TGFβ family proteins, but contains only two instead of three follistatin domains[115]. Several other proteins, containing 1-10 follistatin domains, like the extracellular matrix-associated proteins SPARC and agrin, on the other hand were not able to bind TGFβ family members[100,116]. The FLRG gene was originally identified as a target of chromosomal rearrangement in leukemia[117]. The highest tissue expression of FLRG was found in placenta, whereas highest follistatin expression was found in ovary, testis, and pituitary[115,118]. In HepG2 hepatoma cells, expression of both FLRG and follistatin was induced in response to activin A treatment suggesting that they participate in a feedback loop to restrict activin A signals[119]. FLRG mRNA is up-regulated in rat livers in response to a necrogenic dose of CCl_4 (M.G. unpublished observation) but otherwise the role of FLRG in liver regeneration has not been characterized. Elevated expression of FLRG was found in chemically induced rat liver tumors and H4 ⅡE rat hepatoma cells but not in human liver tumor specimens[62] indicating species-specific differences with respect to FLRG regulation or differences between liver tumors of different etiologies.

Activin receptors

The type Ⅱ activin receptors ActR-Ⅱ (A) and ActR-ⅡB and the type I activin receptors ALK4 and ALK7 are expressed in multiple cell types and tissues including the liver. Adenovirus-mediated overexpression of a dominant-negative type Ⅱ activin receptor caused DNA synthesis and liver growth in normal rat livers[120]. During liver regeneration after partial hepatectomy, no change of ActRⅡ was observed while ActRⅡB was transiently decreased[50]. During CCl_4 induced rat liver cirrhosis, ActRⅡA was reduced after 5 wk but returned to control levels after 10 wk[56]. Ectopic overexpression of ActR-IB (ALK4) and ActR-ⅡB or of ALK7 induced apoptosis in hepatoma cells[121,122]. In HCC tissue specimens, expression of activin receptors (ActR-I, ActR-IB, ActR-Ⅱ, and ActR-ⅡB) was demonstrated by immunohistochemistry[63]. Inactivating mutations of activin receptors have been found in microsatellite instable colon cancer, pancreatic cancer and prostate cancer, but have not been investigated in HCC so far[123-126].

Regulators of activin receptor activity

Several membrane-associated proteins exist which regulate activin-induced receptor activation. Cripto/TDGF1 is a member of the EGF-CFC (epidermal growth factor-Cripto/frl/cryptic) family of growth factor-like molecules. This secreted protein can attach to the outer cell membrane via a glycosylphosphatidylinositol anchor and functions as a co-receptor for nodal signaling during embryogenesis. Cripto has been found overexpressed in high percentages of several human malignancies including breast, pancreas, lung, colon and bladder cancer[127]. Cripto inhibits ligand receptor interactions of activins and TGFβ[128-130] and this has been suggested to contribute to its pro-tumorigenic activity. However, an additional activin receptor-independent signaling pathway for Cripto involving Glypican-1 and c-Src has also been described[127]. Expression of a shorter Cripto variant was observed in colon cancer including liver metastases, as well as in colon cancer and hepatoma cell lines[131,132]. Expression of this short variant is driven by Wnt signaling which is frequently constitutively activated in colon cancer and HCC. Based on these findings, a more extensive investigation on the role of Cripto in HCC is certainly warranted.

BAMBI (bone morphogenetic protein and activin membrane-bound inhibitor) also known as nma (non metastatic gene A) is a pseudoreceptor related to the type I receptors of the TGFβ family. It lacks an intracellular kinase domain and inhibits activin A, TGFβ, and BMP signaling by stably associating with TGFβ family

Table 1 Overview of described alterations of activins and activin antagonists in HCC and HCC-derived cell lines

Activin subunits and activin antagonists	Proposed function in activin signaling	Alterations observed in HCC and hepatoma cells
Activin βA subunit	Activates activin receptors	Increased activin A in circulation of HCC patients[60,61] Decreased expression in rat and human liver tumors[62] Loss of expression in hepatoma cells[74,114]
Activin βE subunit	Induces apoptosis by as yet undefined mechanisms	Decreased expression in rat and human liver tumors[62,85]
Follistatin	Binds activins and blocks their interaction with receptors	Increased in circulation of HCC patients[60] Increased expression in human mouse and rat liver tumors[62,112] Expressed in hepatoma cells[112,114]
FLRG	Binds activins and blocks their interaction with receptors	Increased in rat but decreased in human liver tumors[62]
BAMBI	Binds TGFβ-family type Ⅱ receptors and blocks type Ⅰ receptor activation	Increased in HCC and colon cancer[135]
Cripto	Blocks interaction of activins (and TGFβ) with their receptors	Overexpressed in hepatoma cells[132]
Smad 7	Inhibits activation of Smads by activin (and TGFβ) receptors	Increased expression in HCC[146,147]

receptors[133]. A recent study links LPS/Toll-like receptor 4-induced downregulation of BAMBI in hepatic stellate cells to hepatic fibrosis[134]. In contrast, elevated BAMBI expression driven by the Wnt/β-catenin pathway was found in HCC and CRC specimens[135].

ARIPS 1 and 2 (activin receptor-interacting proteins) are PDZ (PSD-95/Discs-large/ZO-1) protein-protein interaction domain-containing proteins that were described to interact with type Ⅱ activin receptors and inhibit or augment activin signaling, depending on the isoforms expressed[136-138]. ARIP 2 was recently shown to be induced by activin A in the mouse hepatoma cell line Hepa1-6 and to decrease activin-mediated collagen Ⅳ expression, suggesting that it participates in a negative feedback regulation of activin-induced liver fibrosis[139]. Data with regard to a role of ARIPS in HCC or other tumor types are missing so far.

Intracellular inhibitors of signal transduction

Downstream from activin receptors, signals are transduced by receptor Smad 2 and Smad 3 and the common mediator Smad 4, the same set of Smad proteins also used by TGFβ receptors. Mutations of Smad proteins are frequent in pancreatic and colorectal cancer and have also been detected in HCC[140-142]. Smads 6 and 7 associate with TGFβ family receptors but are not phosphorylated and thus inhibit signal transduction[143,144]. Smad7 has been demonstrated to inhibit activin signaling and to protect hepatocytes from activin A-induced growth inhibition[145]. Increased expression of Smad7 has been observed in HCC tissue compared to adjacent tissue[146] and in advanced HCC compared to early HCC or dysplastic nodules[147]. No mutations of either Smad 6 or Smad 7 were found in 52 HCC samples[148].

Smurf-type ubiquitin E3 ligases, Smad anchor for receptor activation (SARA), and transcriptional co-activators and co-repressors such as CBP, p300, c-Ski, and SnoN, control Smad activation by TGFβ-family receptors or shuttling of activated Smads into the nucleus as well as transcriptional activity of Smad-containing complexes[42]. Their role in the link between activin signals and liver carcinogenesis has yet to be defined.

In summary, increasing evidence suggests that deregulation of activin signals frequently occurs in and contributes to HCC development and progression. An overview of alterations in activin subunits and activin antagonists described in liver tumors and hepatoma cells is presented in Table 1.

THERAPEUTIC PERSPECTIVES

Activin signaling is complex. At least three features of the activin signaling cascade contribute to this complexity. First, four activin β and one inhibin α subunit can give rise to multiple homo and heterodimers with different receptor binding capabilities. Secondly, a number of different extracellular activin-binding and receptor-interacting proteins can modulate ligand receptor interactions not only of activins but also of TGFβ, BMPs and GDFs. Thirdly, there is a considerable degree of promiscuity with respect to usage of receptors and intracellular signaling molecules between different members of the TGFβ superfamily[149]. For instance, activins and TGFβ use different type I and type Ⅱ receptors but rely on the same Smad proteins for intracellular propagation of their signals. This makes it a difficult task to dissect their specific contribution to biological activities, especially in tissues such as the liver, where both activins and TGFβ are expressed. In addition, BAMBI, Cripto and Smad7 have all been shown to interfere with signal transduction of activins as well as of TGFβ.

TGF-β1 has a well recognized dual role in carcinogenesis[150]. It acts as a tumor suppressor in early stages of hepatocarcinogenesis by inducing apoptosis and eliminating precursor lesions[151,152]. At a later stage, however, liver tumor cells often become resistant to its proapoptotic effect, and produce large amounts of TGFβ themselves[153]. From the available data on both loss of expression in tumor cells and apoptosis induction[74,114,154], one would postulate that activin A, and possibly activin E, may have a similar tumor suppressive function in the liver as TGFβ. Whether also activins may shift to a pro-tumorigenic function during tumor progression is little explored. For activin A, a contribution to liver fibrosis, enhanced expression of the angiogenic factor VEGF in hepatoma cells, and stimulation of growth and invasiveness of esophageal squamous cell carcinoma cells has been demonstrated[58,63,155].

Despite all the complexity, however, a general theme

in HCC and in other tumor types seems to be the elevated expression of activin antagonistic proteins in the tumor cells, as observed for follistatin, BAMBI, Cripto, and Smad7[62,127,135,146,147]. These may serve to block the growth inhibitory and pro-apoptotic activity of activin A on hepatocytes. Similar observations have been made in additional tumor types, for instance for Cripto in multiple epithelial tumors, BAMBI in colon carcinoma, follistatin in melanoma and FLRG in breast cancer[127,135,156,157].

Consequently, a targeted inhibition of activin antagonists might restore sensitivity to activin-induced growth inhibition and apoptosis, and may thus represent a feasible strategy to inhibit tumor growth. In line with this hypothesis, it has recently been shown that siRNA-mediated silencing of FLRG inhibited breast tumor cell growth *in vitro*, and that monoclonal antibodies to Cripto inhibited growth of testicular and colon cancer cells in xenograft models[128,156]. Future studies will have to clarify whether such approaches may offer new therapeutic opportunities for combating liver cancer.

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