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Review Article

The potential role of MTF-1 in hepatocellular carcinogenesis and neoplastic progression

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Abstract: Humans are constantly exposed to a wide variety of metals, yet high levels of metals are harmful and can even cause cancer. It is well documented that elevated copper might be a hallmark of various malignancies. The liver is the primary organ involved in copper metabolism, and copper has been found to be associated with HCC; however, the underlying mechanism remains elusive. Metal-regulatory transcription factor-1 (MTF-1) plays an important role in maintaining metal homeostasis, including copper homeostasis. Our initial study suggested that copper induces hepatocyte proliferation and activates signaling involved in HCC tumorigenesis and progression. Up-regulation of MTF-1 in HCC cells was consistent with the strong MTF-1 expression observed in HCC tissue microarrays. Following treatment with copper, HCC cells had much higher levels of MTF-1 compared to normal hepatocytes. Hence, it is conceivable that MTF-1 is related to HCC tumorigenesis and progression through its role as a copper-regulating factor. Further investigation into the molecular mechanisms that link increased MTF-1 to transformation would be helpful for targeted HCC treatment.

Keywords: Hepatocellular carcinoma, copper, MTF-1, APE/Ref-1

Introduction

Hepatocellular carcinoma (HCC) is one of the most common types of cancer worldwide [1-3]. In recent years, HCC mortality has not decreased because it is often detected in the later stages, when most treatments are ineffective [4, 5]. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are important in the etiology of HCC. Metastasis from other cancers, such as colorectal, pancreatic, and breast cancer, also contributes to HCC [6]. HCC is highly progressive; although many new chemotherapeutic agents and treatment modalities are now being used clinically, the survival rate has not improved. Thus, there is an urgent need to elucidate the mechanisms controlling HCC tumorigenesis and progression, which will be helpful for the early diagnosis and treatment of HCC patients.

Humans are constantly exposed to a wide variety of metals, such as copper and zinc, from various sources that are important for normal biological functions under homeostatic conditions. However, excess metal levels are harmful and result in toxicity, chronic diseases, and even cancer in humans [7]. The liver is the primary organ involved in the metabolism of copper, a metal that has carcinogenic potential [8]. Excess copper is reportedly a potent oxidizer that produces reactive oxygen species (ROS), which in turn elicit oxidative stress-related cellular disorders, e.g., cancer [9, 10]. Copper accumulation in LEC rat livers led to spontaneous HCC [11]. Conversely, copper-depleted animals developed small, relatively avascular tumors with decreased invasive capacity [12]. Individuals with elevated copper levels are more susceptible to cancer-related mortality, which is consistent with the finding that both serum and tumor copper levels are increased in patients with a variety of malignancies, including HCC [13-16]. In addition, clinical trials with copper chelators have shown promising results [12, 17, 18]. All these findings suggest that cop-

per plays an important role in HCC. In particular, metal homeostasis and detoxification processes are generally controlled transcriptionally by metal-sensing signaling through proteins such as metallothioneins (MTs; MT1 and MT2), which are modulated by metal-regulatory transcription factor-1 (MTF-1) [19, 20]. Thus, by acting as a copper-regulating factor, it is conceivable that MTF-1 is related to HCC tumorigenesis and progression. However, the detailed mechanism requires further investigation.

MTF-1

MTF-1 is a transcription factor that functions by maintaining metal homeostasis and protecting against injury due to excess metal. The protein is structurally and functionally conserved in *Drosophila*, the puffer fish *Fugu rubripes*, mice, and humans; this conservation indicates that MTF-1 plays an important role in maintaining metal homeostasis across species [21-28]. MTF-1 has an N-terminal region that appears to be essential for optimal Mt1 gene activation; a C-terminal region with a modular transcription activation domain; and six zinc fingers that form the DNA-binding domain. Heavy metals, redox stress, growth factors, and cytokines all induce MTF-1. Activated MTF-1 binds to MRE (metal response element) sequences within target promoters, thereby either inducing or repressing target gene expression [29-34]. The reported MTF-1 targets are critical for metal homeostasis, embryogenesis and hematopoiesis [21, 29, 35].

Different mechanisms have been proposed for how MTF-1 detects metals. MTF-1 is activated by serine and tyrosine phosphorylation, the levels of which are regulated by metals [36]. However, MTF-1 phosphorylation levels and overall modification patterns do not change rapidly in response to metals. Recombinant MTF-1 was shown to be an in vitro substrate for casein kinase II (CKII), c-Jun N-terminal kinase (JNK) and protein kinase C (PKC), all of which are in the signaling pathway downstream of the metal-dependent recruitment of MTF-1 to the MT-I promoter. When CKII, JNK and PKC were inhibited, MTF-1 expression did not change significantly, suggesting that these kinases may act through MTF-1 cofactors to regulate metal-activated gene expression. MTF-1 has RNA-binding properties that help control stress-related cell survival pathways at the post-transcriptional level [37]. Previous studies have reported that MTF-1 directly senses and binds free intracellular Zn2+ and subsequently binds to DNA [29, 38]. Interactions with other transcription factors (NF-κB, HIF-1, and SP1) and post-translational modifications of MTF-1 likely influence its targets and transactivational activity [29, 30, 36, 39-42]. Translocation of MTF-1 after zinc exposure is also important. MTF-1 predominantly diffuses in the cytoplasm of resting cells, but it translocates to the nucleus following exposure to zinc [43, 44]. A similar two-dimensional translocation pattern of MTF-1 was observed in both untreated and zinc-treated cells, indicating that cytoplasmic and nuclear MTF-1 share similar modifications. In addition, MTF-1 phosphorylation may contribute to the nuclear translocation of activated MTF-1 [36, 40, 45].

Additionally, MTF-1 was reported to play critical roles in both extracellular matrix remodeling and experimental tumorigenesis [36, 39, 46-48]. As MTF-1 activity is modulated by metal ions, the presence of oxidants, hypoxia, and cytokines facilitates tumorigenic phenotypes, such as improved cell survival, tumor angiogenesis, and the establishment of pro-tumorigenic microenvironments, including inflammatory tumor cell signaling and growth factor independence [47-53]. Accordingly, there is much focus on the role of MTF-1 in tumorigenesis and development.

MTF-1 signaling in HCC carcinogenesis and progression

MTF-1 directly induces the expression of several genes, such as MTs (MT1, MT2A) and Zn transporter-1 (ZNT1/SLC30A1) [20, 21]. MTs protect cells against oxidative stress and contribute to pathways involved in proliferation, survival and energy generation in normal cell types [54-59]; these proteins likely function in a similar manner in tumor cells. Tissue hypoxia and oxidative stress are common features of most solid tumors, including HCC [60]. MTF-1 levels were reported to be significantly increased in breast, lung and cervical carcinomas [61], highlighting the role of MTF-1 in tumor progression. Over-expression of MTF-1 was observed in human intrahepatic cholangiocarcinoma and contributed to tumor differentiation,

vascular invasion, and poor prognosis [62]. Loss of MTF-1 expression inhibited development tumor due to increased matrix collagen deposition and decreased vascular density [46]. In addition, MTF-1 target genes are involved in apoptosis resistance, invasion, metastasis, and angiogenesis and are correlated with tumor progression and aggressiveness. These target genes, including PGF, HIF-1 and TGFβ1, are either up-regulated or induced in multiple human tumors and correlate with tumor progression and disease recurrence [30, 32, 33, 46-48, 63-72]. PGF is a member of the VEGF family and has been associated with cancer stage, survival, invasion, metastasis and recurrence [66, 67]. HIF-1 plays an important role in regulating the cellular response to hypoxia and various signaling pathways involved in tumorigenesis [73, 74]. In several human tumor types, elevated HIF-1α levels have been associated with a high risk of mortality [71, 74]. Moreover, TGFβ1 and TGM contribute to tumor survival and metastasis [67, 69].

Interestingly, *Drosophila* MTF-1 (dMTF-1) plays dual roles to maintain copper homeostasis: it activates copper importer genes to increase copper intake at low copper concentrations and induces MTs to chelate excess copper at high copper concentrations. In these processes, dMTF-1 binds to MREs in the enhancers of target genes [75, 76]. However, in mammals, MTF-1 may have more complicated biological functions. Conditional knockout of MTF-1 did not influence mouse growth or maturation to adulthood, although the mice were extremely sensitive to metal-based toxicity [35]. Knockout of the MTF-1 gene in mice resulted in impaired liver development at gestational day 14 and led to liver decay, generalized edema, and embryonic death, suggesting a critical role of MTF-1 in liver-specific developmental gene expression [77]. In particular, our study confirmed that MTF-1 was up-regulated in HCC cells, consistent with the strong MTF-1 expression observed in HCC tissue microarrays. Following copper treatment, HCC cells exhibited much higher levels of MTF-1 compared to those in normal hepatocytes. Therefore, MTF-1 overexpression in HCC may be an important event in HCC progression and a good candidate for targeted molecular therapy.

Studies to date have emphasized the role of MTF-1 in the basal and induced expression of MT. However, cultured cells from MTF-1 knockout mice lack MT gene expression under both basal and metal-induced conditions [19, 20]. Moreover, the function of MTF-1 may not involve Mt1 or Mt2 because embryonic lethality does not occur in mice null for both these proteins [78, 79]. As a consequence, it is necessary to assess other factors that mediate or interact with MTF-1 in HCC. Apurinic/apyrimidinic endonuclease/redox effector factor 1 (APE/Ref-1) is a potential candidate because it is an important mediator and potentiator of HCC progression [80]. APE/Ref-1, a master regulator of cellular responses to oxidative stress conditions, has been shown to affect tumor progression by transactivating numerous transcription factors involved in cell proliferation, apoptosis, and metastasis, such as AP-1 and nuclear factor-κB (NF-κB). Furthermore, emerging evidence indicates that APE/Ref-1 is elevated in various types of cancer and that its sub-cellular distribution is closely correlated with tumor aggressiveness, resistance to radiotherapy, and poor outcome. We showed that APE/Ref-1 stimulates cellular proliferation, enhances survival, and facilitates metastasis. Compared to cultured normal hepatocytes, HCC cells exhibited higher levels of APE/Ref-1 and ROS stress. Treatment of hepatocytes with copper resulted in transcriptional activation of APE/Ref-1 and induction of downstream targets, whereas cotreatment of HCC cells with copper and the copper chelator disulfiram (DSF) reduced the expression levels of APE/Ref-1, AP-1/c-Fos, matrix metalloproteinase-1 (MMP-1), and Bcl-2. Data from a human HCC tissue microarray indicated that greater cytoplasmic accumulation of APE/Ref-1 was correlated with poorly differentiated and more aggressive tumors, though both nuclear and cytoplasmic APE/Ref-1 signals were significantly higher in HCC than in normal liver tissue. Therefore, the data define a novel role for APE/Ref-1 in HCC progression as an important mediator and potentiator. Interestingly, our immunoprecipitation data established that MTF-1 and APE/Ref-1 interact with each other. As a result, in the liver, MTF-1 and APE/Ref-1 may act in concert in tumorigenesis and progression. Further studies are needed to address these hypotheses.

In conclusion, the role of MTF-1 in hepatocarcinogenesis remains unclear and requires further investigation. However, MTF-1 could be a novel therapeutic target for manipulating metal and/or redox homeostasis in HCC.

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Disclosure of conflict of interest

None.

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