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Review

Serine peptidase inhibitor Kunitz type 2 (SPINT2) in cancer development and progression

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ABSTRACT

Understanding the molecular basis and mechanisms involved in neoplastic transformation and progression is important for the development of novel selective target therapeutic strategies. Hepatocyte growth factor (HGF)/c-MET signaling plays an important role in cell proliferation, survival, migration and motility of cancer cells. Serine peptidase inhibitor Kunitz type 2 (SPINT2) binds to and inactivates the HGF activator (HGFA), behaving as an HGFA inhibitor (HAI) and impairing the conversion of pro-HGF into bioactive HGF. The scope of the present review is to recapitulate and review the evidence of SPINT2 participation in cancer development and progression, exploring the clinical, biological and functional descriptions of the involvement of this protein in diverse neoplasias. Most studies are in agreement as to the belief that, in a large range of human cancers, the *SPINT2* gene promoter is frequently methylated, resulting in the epigenetic silence of this gene. Functional assays indicate that SPINT2 reactivation ameliorates the malignant phenotype, specifically reducing cell viability, migration and invasion in diverse cancer cell lines. In sum, the *SPINT2* gene is epigenetically silenced or downregulated in human cancers, altering the balance of HGF activation/inhibition ratio, which contributes to cancer development and progression.

1. Introduction

Hepatocyte growth factor (HGF) signaling plays an important and well documented role in cancer development and progression. HGF binds to and activates the transmembrane cell-surface receptor c-Met, triggering a signaling network involved in cell proliferation, survival, migration and motility through PI3K/AKT/mTOR, MAPK, FAK and RAC1 signaling [1]. HGF is produced and released as an inactive form, pro-HGF, which requires a site-specific cleavage to be converted into bioactive HGF. The enzyme denominated HGF activator (HGFA) is a serine protease responsible for cleaving pro-HGF into the active form, HGF [2,3]. The serine peptidase inhibitor Kunitz type 2 (SPINT2), also known as bikunin, DIAR3, HAI-2, HAI2, Kop or PB, binds to and inactivates HGFA, behaving as an HGFA inhibitor (HAI) and impairing bioactive HGF production [4]. Thus, activation of the HGF/c-MET axis is closely associated with the balance and availability of pro-HGF, HGFA and HAI (*e.g.* SPINT2) (Fig. 1).

The specific *in vivo* physiological functions of SPINT proteins remain under investigation. The serine peptidase inhibitor Kunitz type 1 (SPINT1; also known as HAI-1), presumably has a role in survival and regeneration of epithelial cells [5,6]. Moreover, SPINT1 appears to be essential for placental differentiation, embryonic development and postnatal survival [7]. SPINT2 is a potent inhibitor of several serine proteases, participating in the transport and shedding of the protease to the cell-surface and, consequently, activating a range of cellular functions [8]. The SPINT2 regulatory mechanisms of matriptase, prostasin, plasmin, kallikrein and pancreatic trypsin are related to degradation and digestive processes, protein processing (such as casein production during lactation), intestinal epithelial homeostasis, tissue remodeling and development [9,10,11,12]. In mice, the loss of SPINT2 results in placental morphogenesis abnormalities and failure, neural tube closure defects and, consequently, embryonic lethality [13]. In humans, SPINT2 apparently has a role in intestinal ionic homeostasis, mainly in sodium absorption [14], and mutations in the *SPINT2* gene are associated with congenital sodium diarrhea [15,16].

In a previous review, Kataoka et al. [17] inspected the contents of HGFA and HAI in normal and pathological conditions, the main focus of their review however, was on SPINT1 (also denominated HAI-1). In 2010, Parr et al. [4] made a summation of HGF inhibitors as potential therapeutics in cancer, and included some findings on SPINT2.

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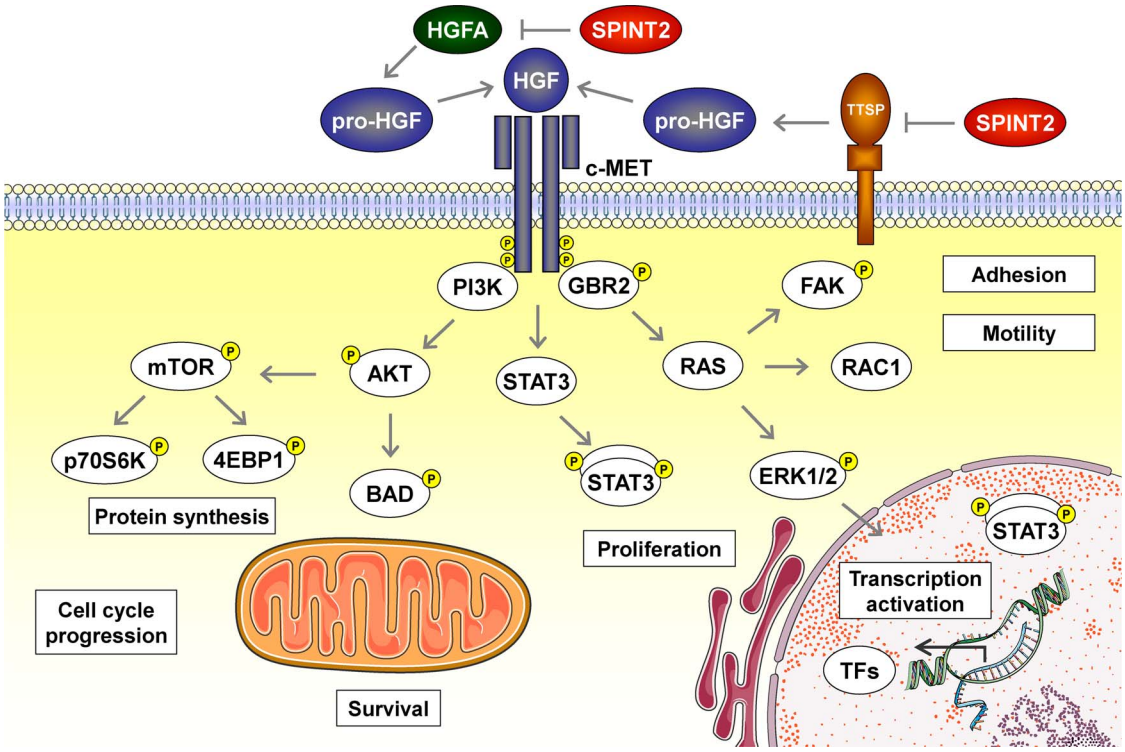

Fig. 1. SPINT2/HGF signaling. Pro-HGF is converted into bioactive HGF by HGFA and/or type II serine proteases (TTSP) (*e.g.* matriptase and hepsin), which is inhibited by SPINT2. Bioactive HGF binds to and activates c-MET receptor, and results in activation of multiple signaling pathways, including PI3K/AKT and MAPK. The activation of HGF signal and its downstream target leads to modulation of transcription activation programs, protein synthesis, survival, proliferation, adhesion and motility. These molecular and cellular alterations contribute to malignant phenotype of cancer cells. A summary of the mainly activated protein network by SPINT2/HGF signaling is illustrated. Abbreviation: P, phosphorylation. This figure was created using Servier Medical Art tools (http://www.servier.com).

However, in recent years, many studies containing relevant information on SPINT2 in cancer biology and tumor microenvironment, as well as, the impact of this inhibitor on clinical outcome have been continuously reported in different types of cancer. In this way, an update on the progress of understanding the functions of SPINT2/HAI-2 may pave the avenues for new insights into this protein in the field of oncology.

In the present review, we analyze and recapitulate the evidence of SPINT2 in cancer development and progression, exploring the clinical, biological and functional descriptions of the involvement of this protein in neoplasias.

2. SPINT2: gene and protein organization

The cDNA sequence of SPINT2 was cloned and characterized by Kawaguchi et al. [18] and Marlor et al. [19] from samples from MKN45 cell line and human placental library, respectively. Currently, the entire *SPINT2* gene is well defined as having approximately 48.5 Kb (start: 38,244,035 bp and end: 38,292,614 bp; orientation: Plus strand), located at 19q13.2 and containing 7 exons. The *SPINT2* cDNA contains 2 transcript variants: the transcript variant *a* is the longer transcript (1.8 Kb), while transcript variant *b* lacks an alternate in-frame exon in the 5′ coding region (1.6 Kb), which results in the lack of an internal segment near the N-terminus of the protein.

SPINT2 protein isoform *a* consists of 252 aminoacids and contains two Kunitz inhibitor domains, which are responsible for the inhibitory activity of trypsin-like serine proteases, while SPINT2 protein isoform *b* consists of 195 aminoacids and contains only one Kunitz inhibitor domain. Both isoforms inhibit HGFA, being the isoform *a* predominant in humans and the isoform *b* predominant in mice [20,21]. The primary structure of SPINT2 protein is illustrated in Fig. 2. SPINT2 is synthesized as a cell surface transmembrane glycoprotein and is secreted after proteolytic cleavage at the juxtamembrane part of the protein [4]. The secreted form, but not the transmembrane form of SPINT2, is capable of complexing and inhibiting HGFA [17]. In addition to its role as an HGFA inhibitor, SPINT2 has also been reported to inhibit the activity of matriptase, hepsin, trypsin, plasmin, tissue and plasma kallikreins, prostasin and coagulation factors IXa, Xa, XIa and XIIa [4,19,22,23,24,25,26]. Of note, some type II serine proteases (TTSP) are also capable of converting pro-HGF to biologically active HGF, triggering HGF/c-MET pathway and participating in cell growth, angiogenesis, and invasion [25,26,27,28,29]. Alterations in TTSP expression and/or activity regulated by SPINT2 (*i.e.* matriptase and hepsin) have been reported in several tumor types, which mediate signal transduction between tumor cells and its surrounding microenvironment (Reviewed by Tanabe and List [30], Murray et al. [31] and Webb et al. [32]).

3. SPINT2 expression and methylation in cancer

Parr and Jiang [33], using semi quantitative PCR, initially demonstrated that SPINT2 is expressed in various human cancer cell lines, including prostate cancer, breast cancer, colorectal cancer, pancreatic cancer, bladder cancer, melanoma, lung carcinoma, hepatocellular carcinoma, fibroblasts and epithelial cells. Screening studies using cDNA microarray were pioneering in the identification of SPINT2 low expression in solid tumors and opened new rounds of investigation of this protein in cancer. These findings have been widely validated by different research groups, as detailed below.

3.1. Neoplasms of liver

Fukai et al. [34] demonstrated that promoter region of *SPINT2* is frequently hypermethylated in hepatocellular carcinoma (21/26, 80%) compared to non-tumor adjacent tissues (7/26, 27%) and normal liver tissue (0/7, 0%), which is associated with *SPINT2* epigenetic gene silencing. In addition, three out of six hepatocellular carcinoma cell lines