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Review Article

The role of human pituitary transforming gene-1 in transcriptional and cytoskeletal regulation of cancer cells

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ABSTRACT

Human pituitary tumor-transforming gene-1 (hPTTG1) is an oncogene that is expressed at a high level in most tumors, especially in metastatic ones. Accumulating evidence reveals that hPTTG1 is a trancription factor that has transcriptional activity either by directly or indirectly binding to DNA. Furthermore, hPTTG1 has been identified to regulate Rho guanine nucleotide exchange factor-H1 (GEF-H1) directly, by an interaction with microtubules and contributes to cancer progression. GEF-H1 activity is important for RhoA-dependent changes in cell morphology and actin organization. hPTTG1 activates GEF-H1/RhoA signaling to affect cytoskeleton organization, cell motility, cell invasion, and breast cancer metastasis. Thus, hPTTG1 links changes in microtubule integrity to RhoA-dependent regulation of the actin cytoskeleton, and therefore promotes cancer metastasis. The molecular mechanism that links microtubule dynamics to RhoA-GTPases has not, as yet, been elucidated.

1. Introduction

Human pituitary tumor-transforming gene 1 (hPTTG1), originally isolated from a rat pituitary tumor GH_4 cell cDNA library [1], has subsequently been identified as a human securin [2] and is a multifunctional protein. In cell cycle progression, hPTTG1 controls sister chromatid separation during mitosis [3,4]. However, hPTTG1 also behaves as an oncogene whose overexpression transforms fibroblasts *in vitro* and enhances tumorigenesis *in vivo*. As assessed by anchorage-independent growth in soft agar, hPTTG1-transfected NIH3T3 and human embryonic kidney (HEK)293 cells formed much larger colonies compared with control vector-transfected cells [1,5–7]. Consistent with these results, H1299 lung carcinoma cells targeted with hPTTG1 small interfering RNA formed smaller colonies in soft agar [8]. *In vivo*, hPTTG1-transfected NIH3T3 or HEK293 cells formed tumors when these cells were injected into nude mice [1,5,7,9]. In several clinical studies, hPTTG1 overexpression has been observed in a wide variety of endocrine and nonendocrine tumors, including those in the pituitary, thyroid, ovary, breast, prostate, lung, esophagus, colon, and central nervous system. In addition, hPTTG1 overexpression is considered a potential invasive marker that is relevant to the malignancy of breast cancer [10]. When analyzing hPTTG1 mRNA expression in 72 primary tumors from patients, a direct correlation was found between hPTTG1 mRNA overexpression and lymph node infiltration [11]. In addition, overexpression of hPTTG1 in breast tumors correlates with a higher degree of tumor recurrence. Taken together, the determination of hPTTG1 expression in primary breast tumors is a powerful tool for the assessment of potential tumor aggressiveness [11]. Tumors that express high levels of hPTTG1 mRNA also exhibit high levels of expression of basic fibroblast growth factor (bFGF), suggesting a correlation between hPTTG1 and bFGF expression, and further suggesting that the hPTTG1 protein may be involved in tumor angiogenesis and mitogenesis [12]. In this review, we describe how hPTTG1 serves as a transcription factor, and its role in directly regulating the GEF-H1 gene and promoting metastasis.

2. hPTTG1 and transcriptional activity

hPTTG1 has one N-terminal basic domain and one C-terminal acidic domain. The C-terminal region of hPTTG1 was found to possess transactivation activity when fused to a heterologous DNA-binding domain [13]. Thus hPTTG1 has been characterized as a transcription factor that regulates the expression of genes involved in a variety of cellular processes [14]. It appears that

Conflict of interest: none.

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hPTTG1 may act by itself, or cooperate with other factors to activate its target genes.

hPTTG1, a transcription factor, can activate several genes such as c-Myc, FGF2, cyclin D3, p21, the sodium-iodine symporter, prolactin, and matrix metalloproteinase-2 (MMP2) [15,16]. This indicates that the transcriptional activity of hPTTG1 may play important roles in a variety of cellular processes. For example, hPTTG1 mediates p21-induced cell senescence and subsequently constrains pituitary tumor development [17]. Several oncogenic transcription factors can promote malignant progression by induction of downstream effectors [18,19]. This concept provides a more plausible explanation of why hPTTG1 is expressed at a high level in most malignant breast cancers.

It was hypothesized that hPTTG1 exerts its metastasis-promoting functions by acting as a transcriptional activator for genes involved in the metastatic process, and found that hPTTG1 directly regulated the transcription of GEF-H1 through hPTTG1-binding element 1 (TTaACT) on the GEF-H1 regulatory sequence [20]. GEF-H1 is an oncoprotein that specifically activates RhoA [21], a well-known member of the Rho family of guanosine triphosphatases (GTPases). Activated RhoA mediates multiple signals that are involved in cytoskeleton organization, cell motility, cell invasion and cell proliferation [22–25]. All of these events contribute to tumor metastasis [26,27].

Furthermore, it is well known that gene regulation can not be determined by only one transcription factor. Thus, an upstream stimulatory factor 1 (USF1) binding motif was found adjacent to hPTTG1-binding element 1. USF1 is the ubiquitous basic helix-loop-helix-leucine zipper transcription factor [28]. From reporter assays, it was verified that USF1 was not required for GEF-H1 transcription with or without hPTTG1. Instead, C/EBP alpha transcription factor may also be a positive regulator for the GEF-H1 gene. C/EBP alpha is a typical basic region-leucine zipper transcription factor [29]. It is expressed at a high level not only in the placenta, lung, colon, and peripheral blood leukocytes but also more frequently in adenocarcinoma cells [30,31]. Thus, C/EBP alpha may contribute to the development of malignancies in a variety of tissues. C/EBP alpha is a transcription factor strongly implicated in myelopoiesis through control of proliferation and differentiation of myeloid progenitors. Recently, several studies have reported the presence of C/EBP alpha-acquired mutations in hematological malignancies [32–34]. Data from reporter assays show that hPTTG1 and C/EBP alpha cooperates to control the activities of the GEF-H1 gene. This indicates that C/EBP alpha may be a coactivator of hPTTG1 for GEF-H1 upregulation in metastatic cancer cells, but this notion should be further investigated.

3. A novel mechanism of breast cancer metastasis

Although there is evidence implicating hPTTG1 in the regulation Myc, MMP2, p21 and p53, how hPTTG1 promotes tumor growth and metastasis through these downstream genes is not clear. Multiple molecular events participate in tumor growth and the metastasis process. It is likely that hPTTG1 regulates different tumor developmental events by different downstream genes. The authors' group was the first, as far as we know, to identify that hPTTG1 can regulate cytoskeletal dynamics through RhoA-GTP activation [20].

RhoA-GTPase is expressed ubiquitously in different human tissues [35]. One important issue in cancer biology is how RhoA-GTPase is activated pathologically. GEF-H1 is the major molecule that regulates the activity of RhoA physiologically. GEF-H1 activity is regulated by microtubule binding [36] and microtubule dynamics that specifically connect cellular RhoA activation and actin cytoskeleton regulation [37]. Consistent with the fact that GEF-H1 mediates RhoA-dependent F-actin stress fiber formation [38,39], results showed that hPTTG1-induced GEF-H1 expression increases the amount of GTP-bound RhoA, thereby increasing the assembly of F-actin stress fibers and focal adhesions in breast cancer cells [20]. GEF-H1 has been proposed to play a role in regulating the metastatic capacities of cancer cells because its regulatory effects on the actin cytoskeleton are likely to enhance the migratory and invasive properties of cancer cells [37]. This speculation is supported by observation of the newly discovered role of hPTTG1/GEF-H1/RhoA signaling in cytoskeleton regulation, which is one of the driving forces in breast cancer metastasis [20].

It is important to emphasize the newly discovered role of hPTTG1 in cytoskeleton control, which is probably pathologically relevant to the potential of tumor cell migration and invasion. Consistent with *in vitro* findings, *in vivo* results demonstrated the importance of hPTTG1/GEF-H1 signaling in driving breast cancer metastasis [20]. In a tail vein metastasis mouse model, it was found that hPTTG1-knockdown MDA-MB-231 cells with GEF-H1 restoration regained their metastatic properties and behaved like wild-type MDA-MB-231 cells. By 10 weeks after tail vein injection, MDA-MB-231 cells in which hPTTG1 was endogenously overexpressed had more metastatic ability (six of six mice developed metastases) than hPTTG1 ectopically overexpressed MCF-7 cells (one of six mice developed metastases; no metastases developed with MCF-7 cells). Perhaps, longer monitoring times are needed to observe these secondary tumors. Therefore, MCF-7 cells (control vector or pcDNA3-hPTTG1) were orthotopically injected into mammary fat pads and it was found that 16 to 18 weeks after injection, hPTTG1-overexpressed MCF-7 cells, but not control MCF-7 cells, formed many large tumor nodules in the lung and pleura [20]. The results indicated that overexpressed hPTTG1 in MCF-7 cells requires a longer time to form tumor metastases compared with MDA-MB-231 cells, which can form tumor metastasis within 10 weeks after injection. Because tumor metastasis is a complicated process that involves, for instance, activated oncogenes and mutated tumor suppressor genes [40], hPTTG1 may need to cooperate with other oncogenic factors to drive metastatic processes in nonmetastatic MCF-7 cells. One possible cofactor for hPTTG1 to drive tumor metastasis is p53 because MDA-MB-231 cells have mutated p53 (R280 K) [41], and MCF-7 cells have wild-type p53 [42]. hPTTG1 has been reported to regulate the transactivation activity of p53 [43]. However, the possible cooperation of overexpressed hPTTG1 and mutated p53 in metastatic progression requires further investigation.

4. The clinical importance of hPTTG1 and GEF-H1 protein in invasive breast carcinoma

Breast tumors are currently evaluated on the basis of several histopathological features including size, grade, and lymph node status, all of which contribute to assessing the overall stage of cancer development. In addition, hormone receptors (estrogen receptor, progesterone receptor) and human epidermal growth factor receptor 2 expression in tumors, together with the histopathological features, are used to classify the progression of the disease and thus currently provide a rational basis for the aggressiveness of treatment.

Ogbagabriel et al proposed that increased hPTTG1 is a marker of breast tumor proliferative capacity and may aid in the detection of cancers more likely to follow an aggressive clinical course, and identification of patients more likely to benefit from adjuvant therapies [10]. It has been demonstrated that low cytoplasmic hPTTG1 expression in normal breast epithelium, whereas abundant nuclear hPTTG1 expression was demonstrated in almost all invasive breast tumor samples (68 out of 70) [20]. Furthermore, abundant

GEF-H1 expression was also detected in almost all invasive breast tumor samples (65 out of 70). hPTTG1 and GEF-H1 overexpression are positively correlated with each other in invasive breast tumor samples. In addition to hPTTG1, GEF-H1 also represents a potential novel marker of breast cancer. Analysis of hPTTG1 and GEF-H1 expression in breast cancer may provide important prognostic insights and portend the response to treatment in breast cancer.

5. Summary

The role of overexpressed hPTTG1 has been identified in regulating breast cancer metastasis [20]. hPTTG1 directly regulates GEF-H1 gene transcription and RhoA activation in breast cancer cells. Additionaly, it has been demonstrated that activation of hPTTG1/GEF-H1 signaling is essential for breast cancer cell migration and invasion, and is required for actin cytoskeleton rearrangement upon RhoA activation. In vivo evidence has shown that hPTTG1/GEF-H1 signaling is required for breast cancer metastasis in a mouse model. Finally, clinical observations showed a positive correlation between hPTTG1 and GEF-H1 expression in human invasive breast cancers. This work has revealed that hPTTG1 activates GEF-H1 as a novel mechanism to regulate breast cancer metastasis via RhoA signaling.

Importantly, there is new insight into how hPTTG1 promotes breast cancer metastasis. Results showed that overexpressed hPTTG1 directly activates GEF-H1, which in turn, activates RhoA, a regulator of actin organisation. hPTTG1 enhances the migratory and invasive ability of tumor cells through RhoA activation. Thus, hPTTG1 is an attractive therapeutic target in treating metastatic cancers.

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