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Review Article

How the overexpressed *hPTTG1* gene promotes metastasis in breast cancer

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ABSTRACT

Human pituitary tumor-transforming gene (*hPTTG1*) is a human securin homolog. It functions as a mitosis regulator during cell cycle progression in normal cells. It is also an oncogenic transcription factor. Deletion of the *PTTG1* gene or its overexpression causes chromosome instability, increasing the probability of tumor formation and metastasis. *PTTG1* has been found to be overexpressed in most human carcinomas with metastatic grade and blastic leukemia. Thus, overexpressed *PTTG1* is suspected to promote tumor metastasis. However, the mechanism by which overexpressed *PTTG1* drives tumor cells into metastasis remains unclear, but it is assumed to occur through activation of its target genes involved in the metastasis processes. Among the *PTTG1*-activated genes, *GEFH1* and *CXCR2* were shown to be directly regulated by *PTTG1*. Molecular biological studies and examination of human tumor samples indicated that overexpressed *PTTG1* promotes tumor metastasis through activation of *GEFH1/RhoA* signaling, resulting in remodeling of cytoskeletal dynamics, enhancing the invasiveness of cancer cells, and driving tumor cells toward metastasis *in vivo*. Overexpressed *PTTG1* can also activate *CXCR2*, which usually induces senescence in cancer cells if the *CXCR2/p21* senescence pathway is functional. However, in most malignant tumor cells in which the *CXCR2/p21* senescence pathway is defective, these tumor cells escape senescence and move toward metastasis. In these cells, overexpressed *PTTG1* directly activates *CXCR2* and its ligands, interleukin-8 (*IL-8*), and *Gro-α* or indirectly activates nuclear factor-κB to activate *IL-8* and *Gro-α*. These cytokines, in turn, recruit inflammatory cells to the tumor sites creating microenvironments that favor tumor metastasis. Thus, *PTTG1*-overexpressed cancer cells can use myriad pathways to execute metastasis. It is important to screen for inhibitors that can neutralize the functions of overexpressed *PTTG1*. These inhibitors could be significant in the therapeutic arsenal against metastatic cancers.

1. Introduction

Perturbation of the genetic constitution of cells disturbs regulatory circuitries leading to the appearance of cancer cells [1]. The genetic changes leading to cancer can occur in sequential steps with mutations, deletions, or amplifications. Eventually, cancer cells break out of the confinement of their adjacent tissues and travel to distant specific sites where they establish new colonies. This disseminated metastasis contributes to the majority of cancer deaths [2]. The genetic, cellular, and molecular bases of metastasis remain elusive. At present, we know at least several well-orchestrated processes are involved in metastasis as follows: (1) activation of epithelial–mesenchymal transition [3], (2) remodeling of extracellular matrix [4], (3) migration of tumor cells to specific secondary sites [5], and (4) neoangiogenesis [6]. It is pertinent and clinically important to identify gene sets or "signatures" so that the mechanisms of metastasis can be illustrated. With advances in genomic studies, knowledge about these genes and pathways involved in tumor metastasis has started to evolve, and sets or groups of genes closely associated with tumor metastasis have been revealed. Human *PTTG1* (*hPTTG1*) is one of the candidate signature molecules for metastasis [7].

hPTTG1, a human securin, was initially isolated from a rat pituitary tumor cell line [8]. Subsequently, its human homolog was isolated from fetal liver and several tumor cell lines [9–11]. *hPTTG1* has a cell cycle-dependent expression pattern [12]. It functions as securin protein [12]. Like vertebrate securin, hpttg1 is subject to ubiquitin-mediated degradation at the end of metaphase [12]. The *hPTTG1* gene encodes for a small protein of 202 amino acids. Hpttg1 has been detected most abundantly in fetal liver, testis, and thymus,

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with little or undetectable levels in other normal tissues [8,9,11]. *hPTTG1* can transform NIH 3T3 cells that form tumors when transplanted into nude mice [8]. Thus, *hPTTG1* behaves as an oncogene. Indeed, *hPTTG1* is overexpressed in most invasive solid tumors and hematopoietic neoplasia [9,11,13–15]. This has been further confirmed by a microarray analysis of comparative expression profiles between metastatic and nonmetastatic carcinoma from patients [7]. Therefore, *hPTTG1* appears to drive tumor cells into metastasis; however, direct evidence that overexpressed *hPTTG1* promotes metastasis of human carcinoma is still lacking.

The promotion of metastasis by *hPTTG1* may ostensibly be through dysfunction of its degradation, allowing it to accumulate and subsequently exert its metastatic functions. Normally, for a cell to proceed through mitosis, securin (*pttg1*) must be degraded by activated anaphase promoting complex/CDC20 (APC/CDC20) at the onset of anaphase. In metastatic cancer cells, the degradation process may be deficient, resulting in excessive *pttg1* and consequently, induction of chromosome instability and aneuploidy, and cell proliferation. In cells with high levels of hpttg1, the metaphase to anaphase transition is blocked and asymmetrical cytokinesis without chromosome segregation or chromosome decondensation without cytokinesis has been observed [16]. There is frequent chromosome loss in cells depleted of the *hPTTG1* gene [17]. The effect, however, was found to be transient; after 12 passages, the karyotype in *hPTTG1* knockout cells was found to be the same as wild-type cells. This suggests additional compensatory mechanisms for chromosome segregation in human cells [18]. There appears to be a range in the amount of hpttg1 needed to maintain normal chromosomal ploidy. Outside of this range, cells are driven to chromosome instability, increasing the probability of tumor formation and metastasis.

2. Genes regulated by *hPTTG1*

PTTG1 can function as a transcription factor [9,19]. The oncogenes *myc* and *bFGF* were initially identified as its directly regulated genes [20]. Chip-on-chip analysis, however, showed elevated hpttg1 binding to the promoters of 746 genes [21]. The results suggest that hpttg1 regulates a wide range of genes. In addition, hpttg1 can interact with a variety of proteins as well. In addition to separase, hpttg1 was found to be associated with *PBF, PP2A, Ku70/80*, and p53 [22–25].

For an overexpressed *PTTG1* gene to promote metastasis, it presumably can activate genes involved in the metastatic processes. We have identified fibroblast growth factor receptor (*FGFR*), urokinase plasminogen activator (uPA), *CXCR2*, and *GEFH1*, among others that are upregulated in *hPTTG1*-overexpressed cells. FGFR is well known to be involved in angiogenesis, which is important in tumor metastasis [26]. It has been identified as an *hPTTG1* directly regulated gene [19]. uPA, a protease, plays a key role in remodeling of extracellular matrix to promote tumor metastasis [27]. Our studies indicated that *hPTTG1* indirectly activates this gene. *CXCR2*, like *CXCR4* and *CCR7*, is an important G-protein-coupled receptor in chemotaxis, the process adapted by metastatic tumor cells to migrate to specific secondary sites [28]. *GEF* regulates Rho family proteins that control cell migration and invasiveness, and plays an important role in promoting tumor metastasis [29]. Our studies indicated that *GEFH1* and *CXCR2* are the genes directly regulated by *hPTTG1*. Therefore, the elucidation of how these two genes function in tumor metastasis would explain why *hPTTG1* is overexpressed in most metastatic carcinomas.

CXCR2 is activated by multiple CXC chemokines, one of which is interleukin-8 (*IL-8*) [30]. *CXCR2* is overexpressed in the metastatic breast cancer cell line MDA 231, in which *hPTTG1* is overexpressed. Interestingly, *IL-8* overexpression in 231 cells had been reported as well [31]. Thus, in these metastatic MDA 231 cells, there is *IL-8/CXCR2* autocrine signaling. This has significant implications on the tumor microenvironment, given the characteristic *CXCR2* expression on cancer cells, endothelial cells, and tumor-associated macrophages. *IL-8/CXCR2* signaling can go through various pathways. One of these principal pathways goes through activation of protein kinase *B/Akt* [32]. *IL-8/CXCR2* signaling has also been shown to induce the activation of the mitogen-activated protein kinase cascade with the phosphorylation of *Erk1/2* in cancer cells [33]. In addition, *IL-8/CXCR2* signaling has been reported to activate phospholipase C. This in turn can generate diacylglycerol to mobilize calcium and activate protein kinase C [34]. Furthermore, the Src family kinases and focal adhesion kinase were found to be downstream targets of *IL-8/CXCR2* signaling in certain cancer cell lines [35]. *IL-8/CXCR2* signaling was also found to activate members of the Rho family of guanosine triphosphatases, promoting the polymerization of the actin cytoskeleton and retraction of cell cytoskeleton [36]. All these pathways usually result in activation of transcription factors whose activity has been shown to promote cell proliferation, cell spreading, motility, and invasion [37–39]. Our studies showed that *CXCR2* and *GEFH1* were the direct targets of hpttg1, and *CXCR2* and *GEFH1* could go through various pathways to affect metastasis. Therefore, to elucidate the mechanisms of how overexpressed *hPTTG1* promotes tumor metastasis, it is important to demonstrate which pathways *hPTTG1* signals go through to drive tumor metastasis.

3. *hPTTG1* drives breast cancer metastasis through activation of the GEF/RhoA signaling pathway

Using the loss-of-function approach (specific gene knockdown of *hPTTG1*) on the human metastatic breast cancer cell line MDA MB231 (231 for short) and the gain-of-function approach (ectopic overexpression of *hPTTG1*) on the nonmetastatic breast cancer cell line MCF7, we explored how the overexpressed *hPTTG1* gene enhances the motility and invasiveness of cancer cell *in vitro* and promotes cancer metastasis *in vitro*. Through reporter analysis, gel mobility, and chromatin immunoprecipitation analysis, we know *hPTTG1* binds directly to the binding sites on the regulatory sequences of the *GEFH1* gene, thus activating GEFH1, which in turn activates its downstream effecter RhoA. Knockdown of *hPTTG1* or *GEFH1* expression reduces the migratory and invasive abilities of metastatic 231 breast cells. Conversely, ectopic expression of *hPTTG1* or *GEFH1* enhances the migratory and invasive ability of nonmetastatic MCF7 cells. These observed effects were due to activation of the *GEFH1/RhoA* signaling axis by overexpressed *hPTTG1* to modulate and remodel the dynamic of cytoskeletal structures.

Using a mouse model for breast cancer metastasis, we also demonstrated that reducing the expression of *hPTTG1* or *GEFH1* in 231 cells by short hairpin RNA (shRNA) knockdown of these genes would reduce the ability of the cancer cells to metastasize to the lung. Reintroduction of *GEFH1* to the *hPTTG1* knockdown 231 cells restores the metastatic ability of these 231 cells. When *hPTTG1* was ectopically expressed in nonmetastatic MCF7 cells that were transplanted into severe combined immunodeficiency (SCID) mice, the metastatic outcomes did not follow our expectation that overexpressed *hPTTG1* would accelerate metastasis. Instead, proliferation of cancer cells was decreased, tumors that formed were smaller, and progression of metastasis was delayed. These puzzling results were eventually explained to be due to oncogene-induced senescence (see the following section). Nevertheless, the activation of *GEFH1/RhoA* signaling axis by the overexpressed *hPTTG1* was recapitulated in immunohistochemical studies of human tumor samples; in aggressive breast carcinomas, the *hPTTG1* gene expression was positively correlated with *GEFH1* expression. The

detailed descriptions of these studies were summarized previously [40,41].

4. The role of *PTTG1*-activated *CXCR2* in breast cancer metastasis—senescence and inflammation (i.e., tumor microenvironment and metastasis)

Like the oncogenes *myc* and *Ras*, *hPTTG1* frequently induced senescence when it is overexpressed in cells. We found that ectopically overexpressed *hPTTG1* in MCF7 could use at least two pathways to activate *CXCR2* and its ligands, *IL-8* and *Gro-α*. It could directly activate the *CXCR2* signaling or it could activate nuclear factor-κB (*NF-κB*) which, in turn, could activate *IL-8* and *Gro-α* to drive *CXCR2* signaling. *CXCR2* signaling induces senescence through p21 expression. Thus, when *CXCR2*-specific shRNA was used to reduce *CXCR2* expression in *hPTTG1*-overexpressed MCF7 cells, p21 expression concomitantly decreases with cell release from senescence. As mentioned earlier, when *hPTTG1*-overexpressed MCF7 cells were transplanted into SCID mice, much smaller tumors were formed and tumor metastasis to the lung was delayed. If we knocked down the expression of *CXCR2* in *hPTTG1*-overexpressed MCF7 cells and transplanted these cells into mice, both tumor growth and metastasis were accelerated. These results strongly suggest that if the senescence mechanism in tumor cells remains intact, the overexpression of metastasis promoting *hPTTG1* would induce senescence. If the mechanism for enforcing senescence is damaged or defective in tumor cells, the overexpression of *hPTTG1* would drive tumor cells toward metastasis. This provides explanation for the difference in metastatic behavior between 231 MCF7 cells. 231 cells have null functions in *p53* among others, and therefore the maintenance of senescence was lost, whereas in MCF7 cells the functions of *p53* and *p21* are intact and thus senescence is reinforced when *hPTTG1* is ectopically expressed.

Although ectopic expression of *hPTTG1* in MCF cells enforces senescence through *CXCR* signaling, these cells appear to enhance the migratory and invasive abilities of nonmetastatic MCF cells in culture. Experiments conducted in SCID mice showed that the *hPTTG1*-overexpressed MCF cells appeared to trigger an inflammatory reaction that recruited macrophages to create a microenvironment that favored and enhanced the metastatic ability of MCF7 cells. It is very likely that the senescent MCF cells secrete inflammatory cytokines that can attract and recruit inflammatory cells to tumor sites. However, detailed mechanisms remain to be illustrated. The examination of human tumor samples essentially recapitulated what we observed in cell cultures and in mouse model studies. Thus, it appears that overexpression of *hPTTG1* induces genetic stress to trigger a DNA damage response in cancer cells. If the machinery of senescence maintenance, i.e., *CXCR2/p21* signaling, remains intact, then cancer cells remain in a state of senescence with retarded metastasis. However, if *CXCR2/p21* signaling is defective, cancer cells move toward metastasis without hindrance. In addition, the overexpression of *hPTTG1* triggers a DNA damage response, which activates *NF-κB* signaling to induce inflammatory reactions that remodel a microenvironment favoring and enhancing tumor metastasis. The results of these observations have been reported elsewhere [42].

5. Implications

1. Cancer cells activate more than one pathway to promote metastasis. Thus, a therapy that targets a cancer driver gene may work for a short time but cancer cells may develop mutations, and in addition, switch to other pathways. Therefore, it is better to determine how many pathways are activated in a particular cancer type (i.e., determine genetic background to provide information about pathways, which tumor cells use to enhance their metastatic potentials). The therapeutic method can then be designed to target key genes in these pathways simultaneously to eradicate all cancer cells before they are able to develop mutations.
2. In addition to targeting oncogenic pathways or repairing pathways alone, it would be helpful to develop inhibitors that can block the crosstalk between cancers and cells that constitute the tumor microenvironment.
3. Because *hPTTG1* is overexpressed in most metastatic cancer cells and blastic leukemia, providing these cancer cells a driving force for metastasis, it will be very desirable to screen for inhibitors that have high-affinity binding for *PTTG1*. This could interfere or block both the ability of *PTTG1* to activate genes involving tumorigenesis and the interacting proteins of *PTTG1*, which are accomplices in tumor metastasis.

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