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HtrA Serine Proteases as Potential Therapeutic Targets in Cancer

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Abstract

The human HtrA family of serine proteases consists of four members: HtrA1, HtrA2, HtrA3 and HtrA4. Although prokaryotic HtrA proteins are well characterized in their dual roles as chaperones and proteases that degrade misfolded proteins in the periplasm, some members of mammalian HtrA proteins are described as potential modulators of programmed cell death and chemotherapy-induced cytotoxicity. Goal of this review article is to describe the molecular alterations associated with these HtrA serine proteases and how these alterations may be associated with tumor behavior and response to chemotherapy. We will also discuss evidence that chemotherapeutic drugs regulate the expression and activation of HtrA serine proteases and that these proteases contributes to programmed cell death. Finally, we will discuss the potential role of epigenetic therapy in targeting the expression and activation of HtrA serine proteases and the mechanisms by which these proteases enhance cytotoxic effect of conventional chemotherapy.

Programmed cell death is a critical component of chemotherapy-induced cytotoxicity, and a determinant in sensitivity to chemotherapeutic agents. As such, various pathways that regulate programmed cell death have been the focus of intense research interest as therapeutic targets in oncology in order to enhance sensitivity to chemotherapeutic agents or to overcome the problem of resistance [1–4]. Two major pathways that regulate programmed cell death are the extrinsic death receptors-mediated and the intrinsic mitochondrial apoptotic signal pathways which converge upon the activation of caspases and the execution of cell death programs [4]. These pathways are in turn regulated by several pro- and anti-apoptotic proteins [5]. Various agents that promote caspase-mediated apoptotic signaling or agents that antagonize survival signaling are being tested in preclinical and clinical trials to supplement conventional chemotherapy [2]. In this paper, we will briefly review current therapeutic targets in cell death program and describe potential novel therapeutic targets consisting of HtrA serine proteases that interact with caspase-mediated apoptotic pathway and facilitate sensitivity to chemotherapeutic agents.

The effectiveness of current chemotherapy regimens depends on how well anticancer drugs can induce cell death in cancer cells. Accordingly, alterations in programmed cell death or survival pathways have been reported to influence cancer cell response to chemotherapy. Thus, chemoresistance is viewed by some as the result of changes in the finely tuned balance between survival and cell death pathways. In this regard, it is important to note that the relative contribution of apoptotic defects to clinical multidrug resistance is still not completely resolved [6, 7], and tumor microenvironment, genetic constitution of the tumor,

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and alternate cell death programs can have considerable impact on the survival of drug resistant cells [6, 8, 9].

In tumorigenesis, cancer cells acquire a selective growth advantage through a diminished capacity to undergo programmed cell death under growth-restrictive condition, aberrant oncogene activation or genetic injuries. Defects in genes involved in cell death programs are frequently associated with various types of malignancies [10], and consequent insensitivity to cell death signaling is considered a hallmark of cancer cells [11]. Insensitivity to cell death signal is suggested to not only promote carcinogenesis by allowing perpetuation and diversification of genetic alterations and oncogene activations in cancer cells but also facilitate resistance to chemotherapy by increasing the threshold against death signals. Apoptosis, the best-characterized mechanism of programmed cell death, involves targeted proteolysis by activated caspases, and this apoptotic pathway is tightly regulated by the balance of pro-apoptotic and anti-apoptotic signaling cascades [5, 12]. In cancer, activation of anti-apoptotic survival pathways and suppression of pro-apoptotic pathways mitigate apoptosis [11].

Conventional Targets

In general, conventional chemotherapeutic agents achieve greater cytotoxicity in cancer cells by either targeting components of DNA synthesis, DNA damage, or mitotic pathways [13, 14]. Conventional chemotherapeutic agents, such as alkylating agents, anthracyclines, topoisomerase inhibitors, and anti-metabolites, target DNA synthesis pathways, whereas vinca alkaloids, taxane, and epothilone target cell cycle progression by disrupting cytoskeletal components involved in cell division [13, 15]. Other agents, such as retinoids and hormone therapies, target specific types of cancer that depend on particular growth signals [3, 16–18]. In addition to conventional therapeutic agents, newer therapeutic agents that target these pathways are being developed to enhance sensitivity and specificity towards malignant cells [15, 19].

Emerging Targets

In recent years, newer therapeutic agents are increasingly used as a combined therapy to affect greater cytotoxicity in cancer cells by specifically targeting altered biological pathways in particular patient population. These newer targeted therapeutic agents include small molecule inhibitors that targets growth factor signaling pathways (EGFR, MAP Kinases, Ras, Src, IGF1R, VEGFR), survival pathways (PI3K/Akt, NFκB, Survivin, IAPs), HSP90 pathway (17-AAG), and cell cycle proteins (cdk inhibitors, flavopiridol, Ro31-7453, SB715992), monoclonal antibodies that target ErbB1, ErbB2, VEGFR, and other factors that promote proliferation or survival signaling [3, 19–29]. In addition to newer therapeutics, several novel therapeutic agents, such as immunotherapy, cytokines (INFα, INFγ, IL2, anti-TNF, and TRAIL), attenuated oncolytic viruses and viral antigen-targeted therapy (Measles, H-1PV, EBV), BH3 mimetic (ABT-737), epigenetic therapies (TSA, SAHA, CBHA, LBH), and metabolic pathway inhibitors (RAD001, rapamycin) are also being tested in preclinical and clinical trials [3, 21, 30–44].

Apoptotic regulators as targets of novel therapeutics

Targeting apoptosis is a logical strategy for cancer drug discovery [45]. One of the most promising discoveries in the developmental therapeutics is the identification of agents that affect programmed cell death pathways regulated by pro-apoptotic and anti-apoptotic proteins belonging to Bcl-2 family, anti-apoptotic survival proteins belonging to inhibitors of apoptosis proteins (IAPs) family, and pro-apoptotic death receptors and caspases. Bcl-2 is an anti-apoptotic protein and is frequently overexpressed in human malignancies, and its

overexpression is associated with tumor maintenance, progression, and resistance to chemotherapy [46]. Accordingly, several strategies, such as small molecule inhibitor (e.g., HA14-1), antisense oligodeoxynucleotides (e.g., G3139), BH3 peptidomimetics, and Bcl-2 inhibitors (e.g., ABT-737), have been developed to inhibit Bcl-2 [42, 47–49]. Another class of proteins with anti-apoptotic properties are IAPs consisting of XIAP, cIAP1, cIAP2, BIRC1, survivin, ML-IAP, and IAP-like protein 2 [50, 51]. These proteins associate with caspases and prevent them from activation; thus they represent major regulators of apoptosis. Overexpression of IAP proteins has been associated with increased resistance to pro-apoptotic stimuli in various solid tumors and hematologic malignancies. According, various IAP antagonists, such as Compound 11, Compound 3, SM-131, and Compound 8, LBW242, TPI 1396-12, and TWX 024, are being tested in preclinical and clinical trials [52]. Death receptors, which are component of extrinsic apoptotic pathway mediated by death inducing ligands, such as tumor necrosis factor (TNF), TNF-Related Apoptosis-Inducing Ligand (TRAIL), and Fas ligand (FasL), are also targeted as novel therapies. TRAIL receptors are overexpressed in various solid malignancies, and thus represent a valid target opportunity in cancer. Several TRAIL receptor agonist monoclonal antibodies have been developed to target this extrinsic signaling pathway, and therapeutic potential of these antibodies are being evaluated clinical trials [21].

Alternative pathways in programmed cell death

Although disruption of caspase activity is one of the characteristics of most cancers [12], recent studies suggest that caspase-independent cell death programs also play key roles in cellular tumorigenesis [53, 54]. Non-caspase proteases like cathepsins and serine proteases like granzymes induce cell death by targeting either distinct or overlapping caspase substrates [53, 55]. Cell death also occurs through non-apoptotic pathways such as autophagy, and these pathways are being targeted for novel therapy [56]. While the exact mechanisms behind non-apoptotic cell death programs are not fully known, involvement of serine proteases, key tumor suppressors like p53, PTEN, p16^{Ink4a}, and anti-apoptotic proteins such as survivin suggests that there is considerable cross-talk between apoptotic and non-apoptotic pathways of cell death [54]. In particular, emerging evidence support the role of serine proteases in programmed cell death and chemotherapy-induced cytotoxicity [53, 57–64].

The HtrA family of serine proteases

The HtrA family of serine proteases was initially identified in *E. coli* by two phenotypes of null mutants that were unable to grow at elevated temperatures (HtrA for High temperature requirement) [65], or failed to digest misfolded protein in the periplasm (DegP) [66]. Subsequently, homologues of HtrA/DegP have been described in a variety of species, including Gram-negative and -positive bacteria, plants and mammals. These proteins normally contain two conserved core domains, a chymotrypsin-like protease domain, and at least one C-terminal PDZ domain. In contrast to other protease-chaperone systems, HtrA represents the first well-known protein quality control factor that acts in an ATP-independent manner [67, 68].

Until now, four human homologues of *E. coli* HtrA have been identified: HtrA1 (L56 or PRSS11) [69, 70], HtrA2/Omi [71, 72], HtrA3 (PRSP) [73] and HtrA4. All mammalian HtrA proteins, belonging to this family, share a highly conserved chymotrypsin-like serine protease domain and one PDZ domain at the C-terminus [74]. Otherwise, structure of N-terminal regions of mammalian HtrA1, 3 and 4 are distinct from that of HtrA2/Omi: mitochondrial HtrA2/Omi possess a transmembrane anchor, and a large section of the N-terminus is removed by processing, whereas the N-termini of HtrA1, 3 and 4 all contain

predicted signal peptides as well as domains that are recognized as IGF binding and protease inhibitor domains [75]. It is interesting to notice that of the four human HtrA family members, HtrA/Omi2 is the only one with a clear intracellular localization [75].

The HtrA family of serine protease appears to be involved in several important biological mechanisms in mammals, such as growth, apoptosis, arthritis, embryogenesis, neurodegenerative and neuromuscular disorder, and cancer. HtrA1 is the first sequenced member of the human HtrA protein family when it was identified as a gene expressed by normal fibroblasts but not by SV40 transformed counterparts [70]. Subsequently, Hu and collaborators found the same gene during analysis of transcripts overexpressed in osteoarthritic cartilage [69]. HtrA1 has a widespread pattern of expression, and its level in human tissues is modulated both in tissues with different physiological activities [76]. Expression of HtrA1 is also modulated during the proliferating phase of endometrium and during pregnancy [77].

The second member of human HtrA protein family, HtrA2/Omi, was independently identified in a yeast two-hybrid screen for Mxi2-interacting proteins [71], and as a stress-activated protease upregulated in mammalian cells in response to stress [72]. It is localized in the mitochondrial intermembrane space, and it is released into the cytosol in response to apoptotic stimuli [62]. The N-terminal tetrapeptide motif (AVPS) of mature HtrA2 is very similar to those of the mature proapoptotic Smac protein (Second Mitochondria-derived Activator of Caspases) and recognized as IAP-binding motif (IBMs); this fact provides evidence that the two proteins function in a similar way to neutralize inhibitor apoptosis proteins (IAPs) [78]. The role of HtrA2/Omi as a promoter of cell death was confirmed independently by several other groups [63, 79–81]. It was shown that the mature HtrA2/Omi is able to induce apoptosis in human cells both in a caspase-independent manner through its protease activity and in a caspase-dependent manner via its ability to disrupt caspase-IAP interaction [82–86]. HtrA2/Omi is expressed ubiquitously in human tissue, and the proteolytic activity of the protein is substantially upregulated in mouse kidneys following ischemia/reperfusion. Gray et al., who identified HtrA2/Omi using presenilin-1 as bait in a yeast two-hybrid screen, found elevated levels of this protein in the nucleus under conditions of heat shock or ER stress induced by tunicamycin treatment [72].

HtrA3 was discovered initially as a pregnancy-related serine protease (PRSP) identified by DDPCR (Differential Display Polymerase Chain Reaction) to be differentially expressed between implantation and interimplantation sites in the mouse uterus [87]. This protein shares high homology with HtrA1 (56% amino acid similarity), and high levels of its expression were detected in the heart, ovary, testis, pregnant uterus and placenta in the mouse [87]. Mammalian HtrA1 and 3 share identical domain organization including an N-terminal signal sequence (SS), an insulin growth factor binding domain (IGFBP) and a kazal-type S protease inhibitor domain (KI) [73].

The cDNA sequence of the fourth member of the HtrA family, named HtrA4, has been also cloned (GenBank accession no. AK075205) and is mapped to 8p12. No biochemical characterization has yet been reported for this protein, but the high homology in structure and domains with HtrA1 suggests some specific expression of this protein in the placenta [75].

HtrA proteases and cancer

Alterations of HTRA1 in cancer

Meta-analyses of publicly available microarray data from Oncomine.org indicate that HtrA1 is down-regulated and shows allelic imbalance in cancer of diverse origins. Z-score

normalized expression values for HtrA proteases are downloaded from Oncomine website [88], and statistical significance between two groups was analyzed by Student's two-tailed t-test. High-resolution genomic survey of brain tumors by Kotliarov et al indicates allelic loss associated with HtrA1 in glioblastoma compared to astrocytoma, mixed oligoastrocytoma, and oligodendroglioma (Table 1, Ref. 97). HtrA1 expression is also downregulated in medulloblastoma compared to normal cerebellum, atypical teratoid/rhabdoid tumor, or primitive neuroectodermal tumor (Table 1, Ref. 98). HtrA1 expression is down-regulated in tumor stem cells derived from glioblastoma cultured in bFGF and EGF compared to serum-cultured cell lines (Table 1, Ref. 99). In addition, alterations in HtrA1 are also associated with specific tumor behavior. For example, greater allelic loss of HtrA1 in glioma is associated with poor prognosis and with increasing grade of tumors (Table 1, Ref. 97).

Several studies indicate that HtrA1 expression is also down-regulated in epithelial ovarian tumors and metastases compared to normal ovary or normal ovarian surface epithelium (Table 1, Refs. 100–103). Consistent with these results, expression analyses by our research group using real-time PCR, RT-PCR, Northern blot analysis, Western blot analysis, and immunohistochemical analysis of tissue microarrays all indicate that HtrA1 is down-regulated in ovarian cancer of different histologies [64, 89]. Moreover, we also identified epigenetic silencing and allelic loss as potential mechanisms associated with HtrA1 down-regulation in ovarian cancer [89]. We also showed that HtrA1 expression is regulated by chemotherapeutic drugs. Most importantly, we showed that expression of HtrA1 primary tumors correlates with better response to cisplatin-based chemotherapy in ovarian cancer and gastric cancer [64]. HtrA1 is activated during drug treatment *in vitro*, and active HtrA1 increases caspase 3/7 activity and participates in chemotherapy-induced cytotoxicity [64].

In breast cancer, several studies indicate that HtrA1 expression is lower in ER-negative tumors compared to ER-positive tumors and that down-regulation of HtrA1 is significantly correlated with higher Elston grade of breast carcinoma (Table 1, Refs. 108–111, 112–115, 109). Moreover, poorly differentiated breast tumors and those with mutant p53 or with lymphocytic infiltration have significantly lower levels of HtrA1 expression (Table 1, Ref. 112).

Microarray studies indicate that HtrA1 is down-regulated in prostate cancer compared to normal controls (Table 1, Ref. 118). In detail, HtrA1 appear to be down-regulated in metastatic prostate cancer compared to localized prostate cancer or normal controls and in hormone-refractory metastatic prostate cancer compared to prostate carcinoma or benign prostate (Table 1, Refs. 118, 120–121) but is upregulated in prostate tumors post neoadjuvant therapy compared to prostate tumors that did not receive neoadjuvant therapy (Table 1, Ref. 119).

HtrA1 is also down-regulated in malignant melanoma compared to benign melanocytic lesions or normal controls (Table 1, Ref. 123). These results are consistent with our studies indicating that HtrA1 is down-regulated in metastatic melanoma lesions compared to primary melanoma lesions from the same patients [90]. Moreover, ectopic expression of HtrA1 in melanoma cell lines inhibited cell growth [90].

In hematologic malignancies, genome-wide analysis of genetic alterations in acute lymphoblastic leukemia indicates that ETV6-RUNX1 positive precursor-B ALL in remission and B-ALL with Pseudodiploidy in remission have higher levels of HtrA1 compared to ETV6-RUNX1 Positive Precursor-B ALL and B-ALL with Pseudodiploidy without remission (Table 1, Ref. 117). Finally, HtrA1 is also down-regulated in metastatic sarcoma compared to primary sarcoma (Table 1, Ref. 122). Interestingly, recent data from

our research group indicates that HtrA1 is down-regulated in lung carcinomas and in mesotheliomas [91, 92].

Collectively, these results highlight the important role of HtrA1 down-regulation in the cancer progression and in the acquisition of aggressive tumor traits in tumors of diverse origins, and provide a rationale for targeting this protein for cancer therapy due to its pro-apoptotic, anti-growth properties and its regulation by therapeutic drugs.

Alterations of HtrA2/Omi in cancer

HtrA2 expression in tumors is somewhat variable according to tumor types. For example, HtrA2 is upregulated in lung adenocarcinoma, superficial or invasive transitional cell carcinoma, and in oligodendroglioma compared to normal controls (Table 2, Refs. 127–128). HtrA2 is also upregulated in squamous cell carcinoma of head and neck compared to normal oral mucosa (Table 2, Ref. 104). Interestingly, HtrA2 is down-regulated in chronic lymphocytic leukemia (CLL) samples compared to normal B-cells (Table 2, Ref. 132). In contrast, studies by Andersson et al indicate that HtrA2 is upregulated in B-Cell Acute Lymphoblastic Leukemia and T-Cell Acute Lymphoblastic Leukemia compared to Acute Myeloid Leukemia (Table 2, Ref. 133), suggesting differential expression of HtrA1 in different types of leukemia. HtrA2 is also upregulated in Wilm's tumors compared to normal fetal kidney or clear cell sarcoma (Table 2, Ref. 125). On the other hand, HtrA2 is down-regulated in renal carcinoma treated with mTOR inhibitor CCI-779 (8 weeks following treatment) compared to untreated renal carcinoma (Table 2, Ref. 134). When comparison was made between microsatellite stable and unstable colorectal carcinoma, HtrA2 is upregulated in microsatellite unstable colorectal carcinoma compared to the former group (Table 2, Ref. 136). However, in ovarian cancer, HtrA2 is consistently down-regulated in ovarian tumor of different histologies (Table 2, Ref. 100). Two independent validation analyses of gene expression in breast cancer by Sorlie et al indicate that HtrA2 expression is down-regulated with increasing tumor staging (Table 2, Refs. 137–138). HtrA2 is down-regulated in lymphoma positive for Ig-Myc translocation compared to Myc fusion-negative lymphoma (Table 2, Ref. 139). In the study by Dhanasekaran et al, HtrA2 is down-regulated in metastatic prostate cancer compared to primary prostate cancer or normal prostate or benign prostate (Table 2, Ref. 140). Finally, in studies by Korkola et al, HtrA2 expression is down-regulated adult male germ cell tumor compared to normal testis (Table 2, Ref. 141). Collectively, the results from these studies indicate that unlike HtrA1 expression in tumors, expression of HtrA2 is variable, upregulated in some tumor types and downregulated in others.

Expression analyses of HtrA3 in cancer

Similar to HtrA2, expression of HtrA3 is variable according to the tumor type. Studies by Hao et al indicate that HtrA3 expression is upregulated in esophageal adenocarcinoma compared to normal or Barrett's esophagus (Table 3, Ref. 142). Similarly, studies by Iacobuzio-Donahue et al indicate that HtrA3 is upregulated in pancreatic adenocarcinoma compared to normal pancreas (Table 3, Ref. 143). Studies by Korkola et al also indicate upregulation of HtrA3 in seminoma compared to normal testis (Table 3, Ref. 141). In contrast, HtrA3 expression is variable in hematologic malignancies depending on specific molecular alterations. For example, studies by Andersson et al indicate that HtrA3 expression is down-regulated in B-Cell Acute Lymphoblastic Leukemia, T-Cell Acute Lymphoblastic Leukemia, and Acute Myeloid Leukemia compared to normal bone marrow (Table 3, Ref. 133). However, in Acute Lymphoblastic Leukemia, studies by Fine et al indicate increased expression of HtrA3 in leukemia positive for MLL/AF4 translocation compared to leukemia positive for BCR/ABL or TEL/AML1 translocation (Table 3, Ref. 145). Studies by Andersson et al also indicate that HtrA3 is overexpressed in acute

lymphoblastic leukemia (ALL) positive for 11q23/MLL or TCF3/PBX1 translocation compared to normal control or ALL with 47–50 chromosomes, BCR/ABL1, Hyperdiploid Greater Than 50, IGH/MYC, or TEL/AML (Table 3, Ref133). In Acute Myeloid Leukemia, studies by Heuser et al indicate that HtrA3 is down-regulated malignancies positive for partial tandem duplication of MLL compared to MLL mutation-negative malignancies (Table 3, Ref. 146). In the study by Fine et al, HtrA3 is upregulated in pro-B acute lymphoblastic leukemia (ALL) compared to Common or Mixed Lineage ALL or Pre-B ALL (Table 3, Ref. 145). Finally, in the study by Schmidt et al, HtrA3 is upregulated in ALL with hyperploidy greater than 50 compared to normal controls or ALL with low hyperploidy, TEL/AML, or t(8:14)(q24:11) (Table 3, Ref. 147). These results highlight the tumor type-specific variation in HtrA3 expression, and as such point to the need to better understand the underlying molecular alterations in cancer before they can be targeted by novel therapeutics.

Alterations of HtrA4 in cancer

Analysis of microarray studies by Sun et al in brain tumors indicates that HtrA4 is upregulated in glioblastoma multiforme compared to control brain from epilepsy patients (Table 4, Ref. 131). Similarly, studies by Richardson et al indicate HtrA4 is upregulated in breast carcinoma compared to normal breast samples (Table 4, Ref. 149). Studies by Varambally et al indicate that HtrA4 is down-regulated in hormone refractory metastatic prostate cancer compared to primary prostate carcinoma (Table 4, Ref. 121). Finally, studies by Maser et al indicate that allelic ratio of HtrA4 is lower in glioblastoma and pancreatic cancer compared to normal DNA control (Table 4, Ref. 148). It should be noted however that these genomic analyses do not indicate specific loss of HtrA4 alleles. Rather, these results indicate a large region of allelic loss in pancreatic tumors. As such, loss of HtrA4 alleles in pancreatic cancer could have been the result of secondary deletion.

Role of HtrA proteins in programmed cell death and chemotherapy-induced cytotoxicity

Studies by Bartling et al suggest that Smac/Diablo released from the mitochondria of lung cancer cells during etoposide treatment was not sufficient to induce apoptosis and may require additional mitochondrial factors such as HtrA2/Omi [93], highlighting the significance of other mitochondrial proteins, such as HtrA2/Omi, in efficient execution of programmed cell death. Cilenti et al reported that HtrA2/Omi expression is regulated by cisplatin in renal cell lines and primary proximal tubule cells [61]. Cisplatin treatment upregulates HtrA2/Omi expression and sensitizes cells to cisplatin. RNAi mediated down-regulation of HtrA2/Omi attenuated cisplatin-induced cytotoxicity. These results suggest a role of serine protease HtrA2/Omi in cisplatin-induced cytotoxicity. Studies by our laboratories also indicate that HtrA1 expression is regulated by chemotherapy [64]. HtrA1 expression is upregulated in ovarian cancer cells following cisplatin or paclitaxel treatment. Moreover, upregulation of HtrA1 resulted in autocatalytic activation of HtrA1 by removal of N-terminal regulatory domain within HtrA1. Active HtrA1 increases caspase 3/7 activity and induces cell death. Consistent with its pro-apoptotic property of HtrA1, down-regulation of HtrA1 in ovarian cancer cells by RNAi resulted in attenuation of chemotherapy-induced cytotoxicity whereas ectopic expression of HtrA1 in null cells resulted in increased sensitivity to chemotherapy-induced cell death [64]. Moreover, higher levels of HtrA1 expression in ovarian and gastric cancer is associated with better response to cisplatin-based chemotherapy in ovarian or gastric cancer patients, highlighting the potential therapeutic value of HtrA1 expression in these cancers [64]. Finally, gene expression analyses by Folgueira et al indicate that gene expression classifier that includes HtrA1 expression is associated with better response to doxorubicin-based therapy in breast cancer [94].

Epigenetic therapies and re-activated HtrA proteins as biomarkers for drug response

Epigenetic therapies targeting reactivation of pro-apoptotic proteins and tumor suppressors that are down-regulated in cancer represent a potential therapeutic strategy to overcome the problem of resistance to chemotherapy or to enhance the efficacy of conventional chemotherapeutic agents [33, 37, 39, 40, 95, 96]. Previous studies indicate that HtrA1 expression may be epigenetically regulated [89]. In ovarian cancer cell line with no expression of HtrA1, treatment with 5-aza cytidine resulted in re-expression of HtrA1 [89]. Our recent unpublished data indicate that HtrA serine proteases contain putative CpG islands in their promoter and exon 1 regions (Fig 1). Furthermore, promoter sequence analysis of bisulfite-modified DNA in ovarian cancer cells indicates that some CpG sites are differentially methylated in non-expressing cells compared to HtrA1 expressing cells (Fig 2A). Consistent with epigenetic as a mechanism of HtrA1 downregulation in cancer, pre-treatment of non-expressing cells with DNA methyltransferase inhibitor, 5-aza-2'-deoxycytidine (DAC) or histone deacetylase inhibitor (LBH589), resulted in re-activation of HtrA1 expression in initially non-expressing cells (Fig 2B). These results suggest that HtrA1 expression is epigenetically controlled in ovarian cancer. Similar analyses in other cancers are underway in our laboratories, and these studies should provide detailed understanding of epigenetic regulation of HtrA1 expression in cancer.

Since HtrA serine proteases are variably down-regulated in specific cancer types by epigenetic mechanisms, they represent potential targets for epigenetic modulation. Future clinical trials involving the evaluation of epigenetic therapies should use epigenetic modification of these genes as therapeutic markers to assess the biological response of epigenetic therapies and should evaluate their prognostic potentials to predict tumor response to combined epigenetic and conventional chemotherapy.

Future approaches

Due to their potential interaction with caspase-mediated cell death pathways, HtrA serine protease pathway represents a potential target of therapeutic intervention. Future studies investigating the cross-talks between caspases and HtrA serine proteases are necessary to fully explore the therapeutic potential of this novel serine protease pathway. In particular, it will be of significant clinical interest in determining the mechanisms by which HtrA expression is regulated by chemotherapy. Detailed understanding of the mechanisms by which HtrA expression and protease activity are regulated is critical before this pathway can be targeted for therapeutic advantage. In addition, the identification of substrates of these proteases will also be essential in gaining insights into how best to target this novel pathway. It is our hope that this review will spark a new interest in taking a fresh look at this novel pathway by researchers of diverse cancer fields, and that combined effort will eventually lead to better and novel approaches to target cancer cells.

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Figure 1. Genomic sequence analysis of the promoter and exon 1 of HTRA genes for the presence of putative CpG islands. Each analysis contains 1000 bases of promoter and entire exon 1. Emboss CpGPlot web-based program with default settings was used to identify putative CpG islands in HTRA genes. Schematic representation of CpG sites, indicated by vertical lines, was obtained from CpG Island Searcher web program.

Figure 2.

DNA sequence analyses of bisulfite-modified genomic DNA corresponding to a putative CpG island in HTRA1 promoter. A) CpG sites are methylated in HtrA1-deficient A2780, but not methylated in HtrA1-expressing SKOV3 cells. Methylated CpG sites (underlined in A2780 sample) were resistant to bisulfite conversion and remained as Cs whereas unmethylated CpG sites (underlined in SKOV3 sample) were converted to Ts. In addition, two single nucleotide polymorphisms were indicated by arrowheads. B) HtrA1 expression is re-activated with 5-aza-2'-deoxycytidine (DAC) or LBH589 treatments in two non-expressing cell lines.

Table 1

Analysis of HTRA1 alterations in cancer.

Tumor Type	Comparison	Z-score Normalized Expression	95% CI	p-value
Brain	Glioblastoma	0.5332	0.4274:0.6390	
(Kotliarov et al) [97]	Oligodendroglioma Malignant	0.9292	0.7889:1.069	
	Oligoastrocytoma Astrocytoma	0.7135	0.3916:1.035	
		0.9038	0.7378:1.07	P<0.0001
Brain	Normal cerebellum	1.193	0.6092:1.776	
(Pomeroy et al) [98]	Atypical Teratoid/Rhabdoid	0.9014	0.3017:1.501	
	Malignant Glioblastoma Primitive	1.75	1.548:1.951	
	Neuroectodermal Medulloblastoma	1.067	0.3858:1.748	
		0.6652	0.5134:0.8170	P<0.0001
Brain	Serum	2.466	2.418:2.513	
(Lee et al) [99]	Serum-free	1.941	1.888:1.995	P<0.0001
Brain	GBM, >5y survival	0.04186	-0.1011:0.1848	
(Kotliarov et al) [97]	GBM, 3–5y survival	-0.04538	-0.2628:0.172	
	GBM, 1–3y survival	-0.253	-0.3765:-0.1295	
	GBM, <1y survival	-0.4041	-0.5003:-0.3080	P<0.0001
Brain	Grade 2	0.9897	0.8675:1.112	
(Kotliarov et al) [97]	Grade 3	0.7385	0.5705:0.9066	
	Grade 4	0.5332	0.4274:0.6390	P<0.0001
Ovarian	Normal Ovary	2.583	2.559:2.607	
(Hendrix et al) [100]	Ovarian Cancer-serous	1.363	1.193:1.532	
	Ovarian Cancer-endometrioid	1.506	1.314–1.697	P=0.0012
Ovarian	Normal Ovary	2.021	1.863:2.179	
(Welsh et al) [101]	Ovarian Cancer	1.203	1.059:1.346	P=0.0034
Ovarian	Normal Ovary	1.955	1.677:2.233	
(Lu et al) [102]	Ovarian Cancer	1.143	0.9547:1.332	P=0.0014
Ovarian	Normal Ovary	2.493	2.320:2.667	
(Adib et al) [103]	Primary Ovarian Cancer	1.679	1.274:2.084	
	Ovarian Cancer Metastasis	1.863	1.496:2.231	P=0.0060
Head&Neck	Normal oral mucosa	1.06	0.9870:1.133	
(Gino et al) [104]	Squamous cell carcinoma	1.325	1.226:1.423	P=0.0018
Tongue	Normal tongue	1.042	0.8885:1.195	
(Talbot et al) [105]	Squamous cell carcinoma	1.585	1.429:1.740	P<0.0001
Lung	Lung adenocarcinoma	0.9551	0.8863:1.024	
(Gordon et al) [106]	Malignant Pl. Mesothelioma	1.726	1.571:1.881	P<0.0001
Breast	Apocrine tumor Basal	1.684	1.462:1.907	
(Farmer et al) [107]	tumor Luminal tumor	1.446	1.305:1.588	
		1.83	1.745:1.916	P<0.0001
Breast	ER+ ER–	1.652	1.607:1.697	

Tumor Type	Comparison	Z-score Normalized Expression	95% CI	p-value
(Wang et al) [108]		1.413	1.329:1.497	P<0.0001
Breast	ER+ ER–	1.539	1.477:1.601	
(Desmedt et al) [109]		1.276	1.185:1.367	P<0.0001
Breast	ER+ ER–	–0.1226	–0.3459:0.1008	
(vandeVijver) [110]		–1.234	–1.669:–0.7998	P<0.0001
Breast	ER+ ER–	2.313	2.218:2.408	
(Chin et al) [111]		1.922	1.763:2.082	P<0.0001
Breast	Lymphocytic infiltration neg	–0.06162	–0.3218:0.1986	
(vant'Veer et al) [112]	Lymphocytic infiltration pos	–2.238	–2.805:–1.670	P<0.0001
Breast	Grade 1	1.786	1.718:1.853	
(Ivshina et al) [113]	Grade 2	1.662	1.606:1.719	
	Grade 3	1.471	1.381:1.560	P<0.0001
Breast	Grade 1	1.248	1.206:1.291	
(Miller et al) [114]	Grade 2	1.17	1.136:1.204	
	Grade 3	1.055	0.9996:1.110	P<0.0001
Breast	Grade 1	0.4186:1.307	0.8626	
(vant'Veer et al) [112]	Grade 2	0.1831	–0.2182:0.5844	
	Grade 3	–1.07	–1.434:–0.7050	P<0.0001
Breast	Grade 1	1.771	1.717:1.825	
(Sotiriou et al) [115]	Grade 2	1.599	1.515:1.684	
	Grade 3	1.543	1.463:1.623	P<0.0001
Breast	Grade 1	1.583	1.487:1.679	
(Desmedt et al) [109]	Grade 2	1.554	1.477:1.631	
	Grade 3	1.309	1.221:1.396	P<0.0001
Breast	p53 wildtype p53	1.21	1.183:1.237	
(Ivshina et al) [113]	mutant	1.055	1.005:1.105	P<0.0001
Breast	p53 wildtype p53	1.711	1.668:1.754	
(Miller et al) [114]	mutant	1.472	1.377:1.568	P<0.0001
Leukemia	B-cell acute lymphoblastic	0.1766	–0.0524:0.4056	
(Raetz et al) [116]	T-cell acute lymphoblastic	0.03617	–0.2615:0.3339	
	T-cell lymphoblastic lymphoma	0.7623	0.5628:0.9617	P=0.0002
Leukemia	Precursor-B ALL (Remission)	0.279	0.05617:0.5019	
(Mullighan et al) [117]	Precursor-B ALL (ETV6-RUNX1 positive)	–0.6435	–0.8601:–0.4269	P<0.0001
Leukemia	B-cell ALL (Remission) B-cell	0.3381	0.08658:0.5895	
(Mullighan et al) [117]	ALL (B-cell ALL with Pseudodiploidy)	–0.8315	–1.143:–0.5199	P=0.0005
Prostate	Normal prostate	1.191	1.022:1.360	
(Welsh et al) [118]	Prostate carcinoma	0.7086	0.6041:0.8132	P=0.0001
Prostate	Prostate cancer	0.0925	–0.0039:0.1889	
(Holzbeierlein et al) [119]	Post neoadjuvant therapy	0.451	0.3350:0.5671	P<0.0001
Prostate	Normal prostate Prostate	0.6178	0.4904:0.7451	
(Yu et al) [120]	carcinoma Metastatic prostate	0.4366	0.3794:0.4938	

Tumor Type	Comparison	Z-score Normalized Expression	95% CI	p-value
	cancer	0.2052	0.1037:0.3067	P<0.0001
Prostate	Benign prostate	1.383	1.266:1.499	
(Varambally et al) [121]	Prostate carcinoma	1.191	1.104:1.278	
	Hormone-refractory metastatic	0.8841	0.6830:1.085	P<0.0001
Sarcoma	Primary	1.755	1.554:1.957	
(Segal et al) [122]	Metastatic	0.96 9	0.7547:1.179	P<0.0001
Melanoma	Normal skin	1.777	1.575:1.979	
(Talantov et al) [123]	Benign nevus	1.247	1.124:1.370	
	Melanoma	0.8427	0.7484:0.9370	P<0.0001
Melanoma	Disomy 3	0.1371	-0.1139:0.3881	
(Tschentscher et al) [124]	Monosomy 3	1.113	0.8287:1.398	P=0.0002
Kidney	Normal fetal kidney Clear	1.069	0.5929:1.544	
(Cutcliffe et al) [125]	cell sarcoma Wilms tumor	1.57	1.258:1.883	
		0.7488	0.6710:0.8266	P<0.0001
Bladder	Normal bladder	1.534	1.466:1.603	
(Sanchez et al) [126]	Bladder carcinoma	1.266	1.176:1.356	P=0.0001

Significant value in each comparison is highlighted by bold.

Table 2

Analysis of HTRA2 alterations in cancer.

Tumor Type	Comparison	Z-score Normalized Expression	95% CI	p-value
Lung	Normal lung	0.5791	0.5250:0.6332	
(Stearman et al) [127]	Lung adenocarcinoma	0.8055	0.7514:0.8595	P<0.0001
Lung	Carcinoid	0.9593	0.8339:1.085	
(Bhattacharjee et al) [128]	Lung adenocarcinoma	0.9483	0.8939:1.003	
Small cell lung cancer		1.178	0.9053:1.451	
Squamous cell lung cancer		0.6416	0.5254:0.7579	P<0.0001
Bladder	Normal bladder	0.5172	0.4563:0.5781	
(Dyrskjot et al) [129]	Superficial carcinoma	0.7156	0.6084:0.8228	
Invasive carcinoma		0.8069	0.6853:0.9284	P=0.0041
Bladder	Superficial carcinoma	0.1761	0.1165:0.2357	
(Stransky et al) [130]	Invasive carcinoma	0.4017	0.3310:0.4723	P<0.0001
Brain	Normal brain	0.9783	0.9350:1.022	
(Sun et al) [131]	Oligodendroglioma	1.116	1.073:1.159	P<0.0001
Head&Neck	Normal oral mucosa	0.1767	0.04534:0.3080	
(Ginos et al) [104]	Squamous cell carcinoma	0.5354	0.4769:0.5940	P<0.0001
Leukemia	Normal B-cells	0.8633	0.7867:0.9399	
(Haslinger et al) [132]	Chronic Lymphocytic	0.5815	0.5457:0.6174	P<0.0001
Leukemia	Acute Myeloid	-0.5786	-0.8323:-0.3249	
(Andersson et al) [133]	B-cell acute lymphoblastic	0.1679	-0.03911:0.3748	
T-cell acute lymphoblastic		0.2809	-0.1135:0.6753	P<0.0001
Sarcoma	Clear cell sarcoma of kidney	0.2173	0.1059:0.3288	
(Cutcliffe et al) [125]	Wilms tumor	0.5851	0.5104:0.6598	P<0.0001
Kidney	Renal cell carcinoma (0wk)	1.053	0.9978:1.108	
(Boni et al) [134]	Renal cell carcinoma (8wk)	0.8908	0.8443:0.9374	P<0.0001
Renal cell carcinoma (16wk)		0.9413	0.8739:1.009	
Breast	GFP-transfected control	0.3907	0.2868:0.4945	
(Bild et al) [135]	H-Ras transfected	0.7573	0.7150:0.7995	P<0.0001
(Human mammary epithelial cells)				
Colon	Stable	0.9233	0.8591:0.9875	
(Watanabe et al) [136]	Instable	1.154	1.072:1.235	P<0.0001
(Microsatellite status)				
Ovary	Normal ovary	0.7325	0.6578:0.8071	
(Hendrix et al) [100]	Ovarian cancer -endometrioid	0.3219	0.2557:0.3882	P=0.0023
Ovarian cancer -mucinous		0.2262	0.1394:0.3130	P=0.0039
Ovarian cancer -serous		0.4153	0.3508:0.4799	P=0.0034
Ovarian cancer -clear cell		0.1361	-0.0295:0.3016	P=0.0040
Breast	Stage 1	0.789	0.5009:1.077	
(Sorlie et al) [137]	Stage 2	0.5419	0.1423:0.9416	

Tumor Type	Comparison	Z-score Normalized Expression	95% CI	p-value
Stage 3		-0.06275	-0.3027:0.1771	
Stage 4		-0.5341	-0.7977:-0.2718	P<0.0001
Breast	Stage 1	0.8231	0.5382:1.108	
(Sorlie et al) [138]	Stage 2	0.5781	0.3016:0.8558	
Stage 3		0.2434	0.08463:0.4022	
Stage 4		-0.0657	-0.2724:0.1410	P=0.002
Lymphoma	Negative	1.175	1.143:1.207	
(Hummel et al) [139]	Ig-Myc	1.024	0.9802:1.067	P<0.0001
(Myc Fusion)				
Prostate	Normal or BPH	1.181	0.8113:1.551	
(Dhanasekaran et al) [140]	Primary prostate cancer	0.5549	0.3905:0.7194	
Metastatic prostate cancer		-0.1939	-0.3891:0.00138	P<0.0001
Germ cell	Normal testis	1.113	1.065:1.161	
(Korkola et al) [141]	Adult seminoma	0.9188	0.8698:0.9678	P=0.0057

Significant value in each comparison is highlighted by bold.

Table 3

Analysis of HTRA3 alterations in cancer

Tumor Type	Comparison	Z-score Normalized Expression	95% CI	p-value
Esophagus	Normal esophagus	-1.896	-2.426:-1.366	
(Hao et al) [142]	Barretts esophagus	-1.152	-1.773:-0.5303	
Adenocarcinoma		0.9189	0.6080:1.230	P<0.0001
Pancreas	Normal	0.2267	-0.5146:0.9681	
(Iacobuzio-Donahue et al) [143]	Pancreatic cancer	2.606	1.822:3.391	P=0.0019
Germ cell	Normal testis	0.2704	0.1583:0.3826	
(Korkola et al) [141]	Seminoma	0.5982	0.5418:0.6546	P=0.0008
Leukemia	Normal bone marrow	2.478	1.958:2.997	
(Andersson et al) [133]	B-cell ALL	-0.3214	-0.820:0.177	
T-cell ALL		-1.843	-2.814:-0.8715	
AML		0.3605	-0.4062:1.127	P=0.0008
Leukemia	Normal	0.285	-0.061:0.631	
(Ross et al) [144]	BCR-ABL	0.7115	0.529:0.893	
Hyperdiploid		0.5671	0.410:0.723	
MLL		1.228	0.9728:1.484	
Pseudodiploid		0.6376	0.2539:0.9549	
T Lineage		0.384	0.2539:0.5142	
TEL-AML		0.3475	0.1059:0.5892	
E2A-PBX		1.36	1.241:1.479	P<0.0001
Leukemia	BCR/ABL	-1.123	-1.68:-0.562	
(Fine et al) [145]	TEL/AML	-1.859	-2.320:-1.398	
MLL/AF4		3.239	2.239:4.239	P<0.0001
Leukemia	Normal	-1.062	-1.97:-0.149	
(Andersson et al) [144]	11q23/MLL	4.146	1.708:6.585	
47-50 Chr		-0.3508	-4.227:3.525	
BCR/ABL1		0.09373	-4.971:5.158	
>50 Chr		-0.7515	-1.537:0.03378	
IgH/Myc		-2.335	-3.186:-1.484	
TEL/AML		-1.106	-1.980:-0.2318	
TCF/PBX1		3.161	2.344:3.978	P<0.0001
Leukemia	Negative	0.09801	-0.1550:0.3511	
(Heuser et al) [146]	Partial tandem dup	-0.7226	-0.9707:-0.4745	
P=0.0012 (MLL mutation)				
Leukemia	Common	-1.493	-1.968:-1.019	
(Fine et al) [145]	Mixed	-1.11	-6.258:4.038	
Pre		-0.9608	-3.202:1.280	
Pro		3.248	2.125:4.370	P<0.0001
(Immunophenotype)				

Tumor Type	Comparison	Z-score Normalized Expression	95% CI	p-value
Leukemia	Normal	−0.08158	−0.188:0.025	
(Schmidt et al) [147]	Low hyperploid	−0.03072	−0.147:0.0855	
TEL/AML		−0.1678	−0.241:−0.0939	
t(8:14)(q24:11)		−0.1595	−0.242:−0.0765	
>50 Chr		0.1393	0.07798:0.2006	P=0.0013
Breast	Negative	−0.3053	−0.5682:−0.04247	
(vant'Veer et al) [112]	Positive	−1.416	−1.747:−1.084	P<0.0001
(Lymphocytic infiltration)				
Brain	Glioblastoma	−0.2522	−0.3295:−0.1750	P<0.0001
(Maser et al) [148]	(DNA copy)			

Significant value in each comparison is highlighted by bold.

Table 4

Analysis of HTRA4 alterations in cancer.

Tumor Type	Comparison	Z-score Normalized Expression	95% CI	p-value
Brain	Normal	-0.6262	-0.7830:-0.4694	
(Sun et al) [131]	Glioblastoma	-0.2154	-0.2858:-0.1450	P<0.0001
Breast	Normal	-0.8074	-0.9453:-0.6695	
(Richardson et al) [149]	Breast carcinoma	-0.4429	-0.5569:-0.3289	P=0.0004
Prostate	Primary	-0.6017	-0.9403:-0.2630	
(Varambally et al) [121]	Hormone-refractory	-1.574	-2.140:-1.009	P=0.0082
Brain	Glioblastoma	-0.272	-0.3410:-0.2029	P<0.0001
(Maser et al) [148]				

Significant value in each comparison is highlighted by bold.