Drugs of the Future 2011, 36(3): 241-247

CCC: 0377-8282/2011
DOI: 10.1358/dof.2011.36.3.1583184

TARGETING HSP 27 FOR THE TREATMENT OF CASTRATION-RESISTANT PROSTATE CANCER

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SUMMARY

Prostate cancer is the most common cancer in men and the second leading cause of death from cancer in North America. While responsive to androgen ablation in its early stages, prostate cancer eventually becomes castration-resistant (CRPC) and metastasizes, preferentially to bone. Once this happens, the disease carries considerable morbidity and is incurable. Previous work conducted in our laboratory showed that heat shock protein HSP 27, a stress-activated cytoprotective chaperone, is upregulated after castration and chemotherapy in prostate cancer and associated with metastasis and poor prognosis. HSP 27 acts through an ATP-independent mechanism, making this target less amenable to inhibition by small molecules, and so strategies to inhibit HSP 27 at the gene expression level become appealing. Indeed, known nucleotide sequences of cancer-relevant genes offer the possibility to rapidly design antisense oligonucleotides (ASO) or short interfering RNA (siRNA) for loss-of-function and preclinical proof-of-principle studies. We will review antiapoptotic and cell survival pathways regulated by HSP 27 and preclinical studies using antisense therapeutics (OGX-427) to support targeting of HSP 27 in CRPC.

INTRODUCTION

Prostate cancer is the most common male cancer in North America and the second leading cause of cancer deaths. More importantly, its incidence and mortality will double by 2020 based on current incidence trends and the aging baby boomer population. While many gains have been made in the early detection and treatment of localized prostate cancer, many men still die of recurrent metastatic disease. Androgen ablation in the form of medical or surgical castration therapy remains the most effective therapy for patients with advanced disease, and while approximately 80% of patients respond initially, they will eventually progress to castration-resistant prostate cancer (CRPC) (1-6). CRPC progression is a complex process by which cells acquire the ability to both survive and proliferate in the absence of gonadal androgens and involves mechanisms attributed to reactivation of the androgen receptor (AR) axis (7), alternative mitogenic growth factors and cytokine pathways (8-10), stress-induced prosurvival genes (11-13) and cytoprotective chaperone networks (14-15). Intriguingly, over 80% of CRPC specimens continue to express the AR and androgen-responsive genes (16), indicating that the AR axis remains inappropriately activated, despite castration levels of testicular and adrenal androgens. Many mechanisms have been postulated to account for aberrant AR activation in CRPC tumors: 1) activation of AR by nonsteroids such as growth factors and cytokines via deregulated multiple signaling pathways involving kinases; 2) genetic mutations or amplification of AR that render the receptor hyperactive, which sensitizes cells towards low levels of androgen; 3) altered expression of or activity of AR coactivators or chaperone proteins; 4) expression of AR splice variants that lack a ligand binding domain and are constitutively active in a ligand-independent manner; and 5) the increase of intracrine androgen. These mechanisms are not mutually exclusive, and indeed, it is likely that they work in concert to induce CRPC. Despite the failure of maximal androgen blockade trials using nonsteroidal antiandrogens such as flutamide or bicalutamide, CRPC tumors are not uniformly hormone-refractory and remain sensitive to therapies directed against the AR axis. Hence, several new classes of AR-targeting agents are now in clinical development, including more potent AR antagonists (e.g., MDV-3100), inhibitors of steroidogenesis (abiraterone) and AR-disrupting agents that target AR chaperones such as heat shock proteins HSP 90 (17-AAG analogues) or HSP 27 (OGX-427).

Molecular chaperones, including HSPs, help cells cope with stress-induced protein misfolding, aggregation and denaturation. They play prominent roles in cellular signaling and transcriptional regula-

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tory networks. Chaperones act as genetic buffers, stabilizing the phenotype of various cells and organisms at times of environmental stress, enhancing the Darwinian fitness of cells during transformation, progression and treatment resistance (17). Indeed, the heat shock response is a highly conserved protective mechanism for eukaryotic cells under stress, and is associated with oncogenic transformation, proliferation, survival and thermotolerance (18). Molecular chaperones have multifunctional roles in endoplasmic reticular, stress and cellular signaling, and transcriptional regulatory networks associated with cancer progression, metastasis and treatment resistance. Therefore, targeting these chaperones is an attractive and rational therapeutic strategy. Of special relevance to treatment-resistant cancers and metastasis are those chaperones such as HSP 27 that function to inhibit cell death and induce cell motility and invasion (14, 19).

HSP 27 STRUCTURE

HSP 27 (or HspB1), and its murine homologue HSP 25, belong to the family of small HSPs, which to date include nine other isoforms designated HspB2-9 (20-23). All these proteins contain a highly conserved region referred to as the α-crystallin domain, which contains β-sheets (Fig. 1). The *N*-terminal WDPF domain is less conserved, owing its name to the presence of the amino acid residues W (tryptophan), D (aspartic acid), P (proline) and F (phenylalanine). The amino-terminal part of the protein also contains the partially conserved sequence PSRLFDQXFGEXLL, while the carboxy-terminal region consists of a flexible, disordered region. The WDPF region of HSP 27 is crucial for oligomerization, but other sequences in the *N*-terminal region are required (21). The α-crystallin domain is also required for HSP 27 oligomerization, while the flexible region likely participates in interaction with target proteins, and is important for solubility (20, 22). HSP 27 can form oligomers to increase affinity for client proteins, preventing their precipitation and aggregation (24). HSP 27 can be phosphorylated on three serines (Ser^{15}, Ser^{78} and Ser^{82}) and on threonine (Thr^{143}). HSP 27 phosphorylation is a reversible process catalyzed by a large number of kinases, including MAPKAP kinases 2 and 3, p90RSK and protein kinases PKC, PKD and PKG. HSP 27 becomes phosphorylated in response to a variety of stresses, including differentiating agents, mitogens, inflammatory cytokines, such as TNF-α, transforming growth factor-beta (TGF-β), insulin-like growth factor I (IGF-I), androgens, interleukin-1β and oxidative stress.

HSPB1 is a single-copy gene covering a 2.2-kb transcript organized in 3 exons encoding a 205-amino-acid protein. HSP 27 transcriptional activation is under the control of heat shock factor proteins HSF 1 and 2, which bind to consensus sequences within the promoter region of the *HSPB1* gene. The HSP 27 promoter contains two functional heat shock element (HSE) binding sites. The first of these occurs approximately −200 bp upstream of the first exon (25), whereas the second occurs within the first intron (26). Recently, an atypical cAMP response element (CRE) was located at −678 bp upstream of the HSP 27 exon 1 (27). This CRE was recognized by both activating transcription factors ATF-3 and ATF-5, leading to the transactivation of the *HSPB1* gene, which was associated with cell survival against stress (27, 28). Several studies suggest that the specific transactivation of HSP 27 is stimulus-dependent. During mitosis, heat shock factor protein HSF 2 binds to the HSE of HSP 27, whereas HSF 1 appears to bind more robustly during hemin treatment (26, 29). Additionally, several hypoxia-inducible factor HIF-1 binding sites were found to be necessary for endogenous upregulation of HSP 27 in retinal neurons subjected to sublethal ischemic stress (30). The transcriptional regulation of HSP 27 thus appears to be under the control of multiple factors among various cell types and cellular states.

HSP 27 REGULATION OF CELL SURVIVAL

HSP 27 is a potent antiapoptotic molecule, interacting with and inhibiting components of both stress- and receptor-induced apoptotic pathways (Fig. 2). HSP 27 prevents activation of caspases by sequestering cytochrome c in the cytoplasm (31). HSP 27 binds to cytochrome c released from the mitochondria to the cytosol and prevents cytochrome c-mediated interaction of apoptotic protease-activating factor 1 (APAF-1) with caspase-9. Cytochrome c interacts with APAF-1 and caspase-9 to form the "apoptosome", which activates caspase-3 and a cascade of downstream caspases, the so-called "effectors" of cell death. HSP 27 interacts with and inhibits caspase-3 activation, an effect related to the ability of HSP 27 to stabilize actin microfilaments (32). HSP 27 binds to filamentous actin to prevent disruption of the cytoskeleton after stress (33), and also the mitochondrial Smac and BID translocation. Moreover, HSP 27 regulates protein kinase Akt and inhibits apoptosis regulator BAX oligomerization and translocation to the mitochondria (34). HSP 27 can inhibit apoptosis induced by etoposide or TNF-α in different cancer cell lines by increasing I-kappa-B-alpha (IκBα) ubiquitination/degradation, leading to the activation of nuclear factor NF-κB (35).

Recently, upstream signaling pathways mediating HSP 27 antiapoptotic activity related to suppression of mitochondrial cell death and protein synthesis have been proposed. These pathways include the

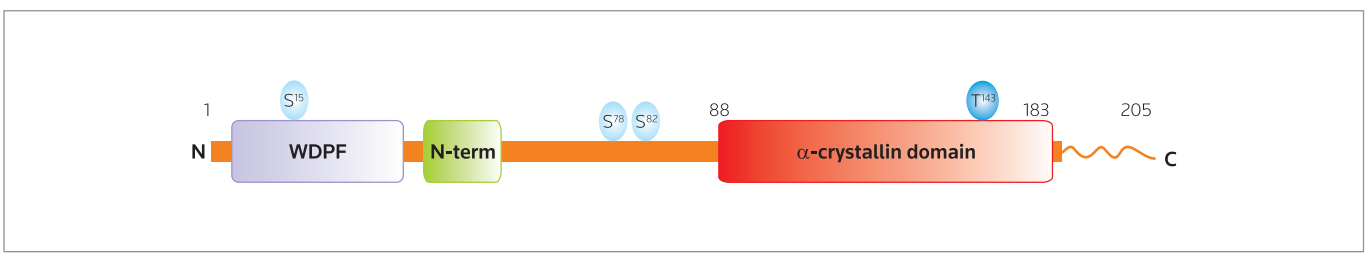

Figure 1. Functional domain of HSP 27 showing the WDPF domain, the *N*-terminal region and the α-crystallin domain. The *zigzag line* corresponds to the flexible domain in the *C*-terminal part of the protein. The phosphorylation sites serine S^{15}, S^{78} and S^{82} and threonine T^{145} are indicated.

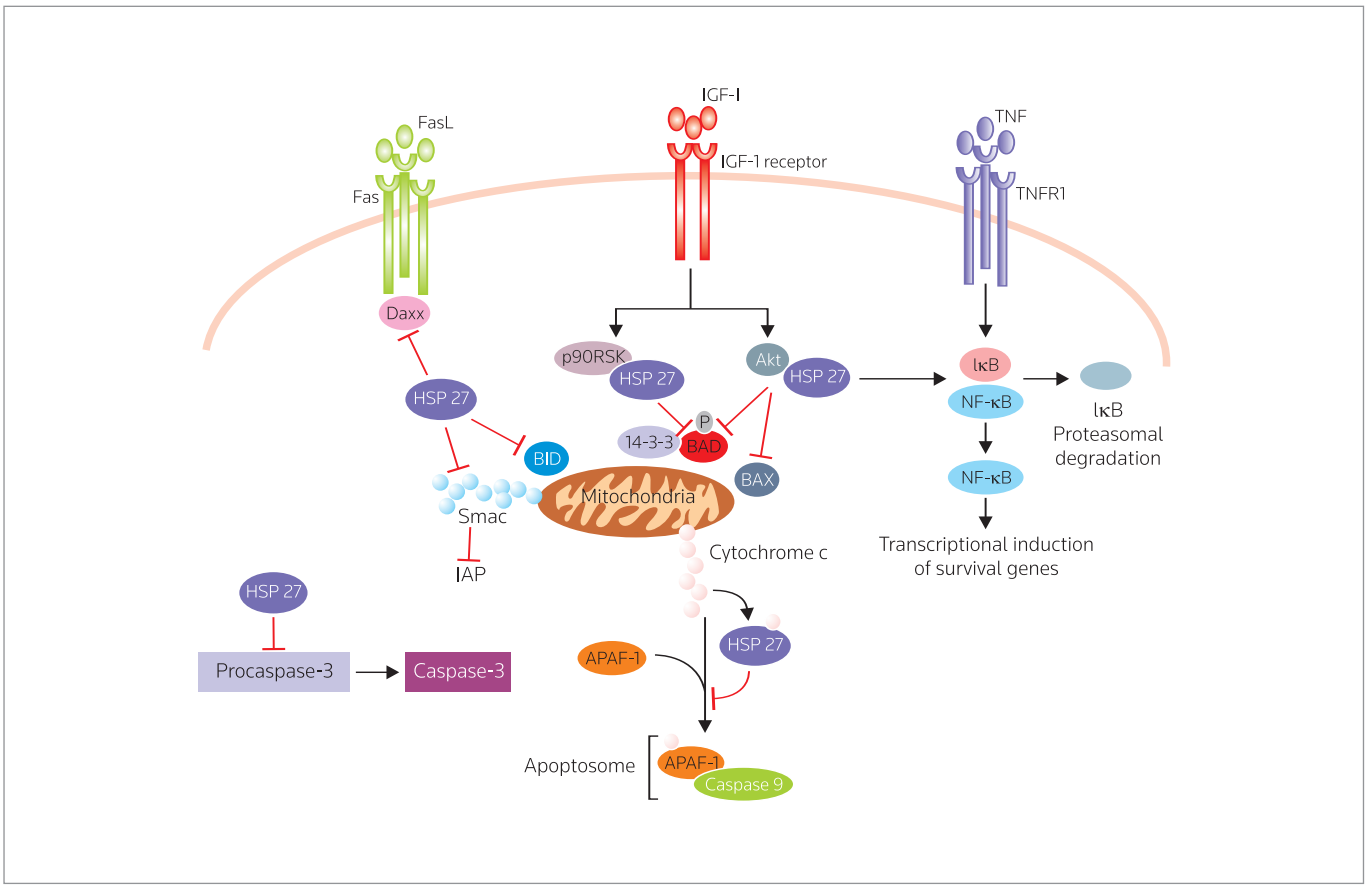

Figure 2. Effect of HSP 27 in suppressing stress-induced apoptosis. HSP 27 inhibits apoptosis by integrating different signaling pathways, including extrinsic and intrinsic apoptosis pathways, as well as growth factor pathways. HSP 27 enhances IGF-I-induced BAD phosphorylation and increased BAD/14-3-3 complexes, thereby preventing BAD mitochondrial translocation. HSP 27 inhibits the extrinsic apoptotic pathway via its ability to block death domain-associated protein 6 (Daxx)-mediated apoptosis by preventing its translocation to the membrane, and thus inhibiting its interaction with FAS and apoptosis signal regulating kinase ASK-1, and enhances TNF-α-inducing I κB proteasomal degradation and activating the nuclear factor NF-κB signaling pathway. HSP 27 negatively regulates the intrinsic apoptosis pathway by inhibiting the activation of procaspase-9 and sequestering cytosolic cytochrome c from apoptotic protease-activating APAF-1 after its release from mitochondria, and thus prevents assembly of the apoptosome. HSP 27 also mediates inhibition of procaspase-3 activation, most likely through its ability to prevent initiator caspases like caspase-9 from gaining access to the residues whose cleavage is essential for procaspase-3 activation.

demonstrated ability of HSP 27 to activate members of the IGF-I signaling pathway and to sequestrate BAD in the cytoplasm by increasing its interaction with 14-3-3 (36) and the ability of HSP 27 to protect eIF4E from ubiquitination and proteasomal degradation (37). For example, HSP 27 overexpression increases IGF-I-induced phosphorylation of extracellular signal-regulated kinase (ERK), p90RSK and Akt, thereby enhancing the BAD/14-3-3 interaction. Conversely, HSP 27 knockdown abrogates IGF-I-induced phosphorylation of ERK, p90RSK and Akt, thereby destabilizing BAD/14-3-3 complexes and increasing apoptotic rates (36).

HSP 27 INVOLVEMENT IN CANCER PROGRESSION AND METASTASIS

Aberrant expression of HSP 27 has been associated with aggressive tumor behavior. Clinically, HSP 27 levels increase in a wide variety of cancers, including breast (38), ovarian (39), glial (40), prostate (41, 42) and others (43). HSP 27 mediates tumorigenesis through inhibition of cell apoptosis, an essential hallmark in cancer progression (32, 44), and is associated with metastasis and poor prognosis (14, 45). For example, HSP 27 is highly expressed in biopsies, as well as the serum of breast cancer patients (46-48). HSP 27 is associated with estrogen-responsive malignancies and correlates with poor prognosis (49). Overexpression of HSP 27 in normal human breast epithelial cells protects from senescence-induced doxorubicin via suppression of p53 activation (50). In murine breast carcinoma 4T1 cells, HSP 27 overexpression enhances tumor metastasis (51). HSP 27 is required for actin rearrangement and cytoskeleton organization and cell migration (52). Its overexpression enhances cell adhesion, migration and invasion via the modulation of focal adhesion kinase (FADK)-dependent actin organization and signal transducer and activator of transcription STAT3-dependent matrix metalloproteinase MMP-2 expression (53). Knockdown of HSP 27 inhibits vascular endothelial

growth factor (VEGF)-induced cell migration and tubulogenesis (54) and abrogates TGF-β, inducing MMP-2 and cell invasion in human prostate cancer cell lines (55, 56).

HSP 27 IS UPREGULATED IN CRPC

HSP 27 expression is induced by hormone and chemotherapy and inhibits treatment-induced apoptosis through multiple mechanisms (19, 36, 45, 57-59). HSP 27 expression and phosphorylation correlate with CRPC progression (19, 36). HSP 27 expression increases shortly after treatment with androgen ablation, and becomes highly uniformly expressed in CRPC (14, 19, 36, 41). HSP 27 expression at diagnosis predicts poor clinical outcome independent of *ETS* gene rearrangement (60).

Consistent with its multiple cytoprotective functions, overexpression of HSP 27 renders human prostate cancer LNCaP tumors resistant to paclitaxel, enhances tumor growth and confers hormone resistance post-castration (19, 37). HSP 27 interacts with factors involved in the oncogenic signaling pathway and prostate cancer progression to CRPC, such as STAT3, IGF-I, AR and eIF4E (19, 37, 61, 62). For example, HSP 27 binds to AR and displaces HSP 90 as the predominant chaperone after androgen treatment, shuttles the AR to the nucleus and facilitates AR binding to the androgen response element in AR-regulated genes (62). Additionally, after hormone therapy, increased HSP 27 confers hormone resistance post-castration via activation of a STAT3-dependent pathway (19), and stabilization of eIF4E by inhibiting its stress-induced ubiquitination and proteasomal degradation and enhancing cell survival (37).

PRECLINICAL PROOF OF PRINCIPLE FOR HSP 27 INHIBITION

Although HSP 27 is a very appealing cancer target, it acts through an ATP-independent mechanism, making it less amenable to inhibition by small molecules, despite the recent publication by Xia et al. (63) showing that a small-molecule triazole ribonucleoside induces apoptosis in pancreatic cancer cells with a decrease in HSP 27 expression levels; however, the mechanism and the specificity of the drug against HSP 27 knockdown remain unclear. For this reason, strategies to inhibit HSP 27 at the gene expression level are attractive. Indeed, known nucleotide sequences of cancer-relevant genes offer the possibility to rapidly design short interfering RNA (siRNA) duplexes (64) or antisense oligonucleotides (ASO) (65).

OGX-427 is a phosphorothioate antisense oligonucleotide complementary to HSP 27 mRNA that incorporates second-generation chemistry with a 2′-methoxyethyl modification to the flanking nucleotides, which enhances tissue half-life and potency. It has been demonstrated by Rocchi et al. (14, 64) that in vitro and in vivo inhibition of HSP 27 expression causes induction of apoptosis with single-agent antitumor activity, as well as enhancement of hormonal therapy and chemotherapy effects when given in combination.

Both unmodified phosphorothioate and the second-generation HSP 27 ASO (OGX-427) have demonstrated anticancer activity in vitro and in vivo (14, 19, 62, 66). We (62) and others (37) showed that an HSP 27 ASO induces AR (62) and eIF4E (37) degradation via the proteasome, leading to decreases in prostate-specific antigen (PSA) expression in vivo (62). OGX-427-mediated HSP 27 knockdown reduces AR and eIF4E stability, enhancing their ubiquitination and degradation, thereby reducing cell viability after androgen withdrawal and/or chemotherapy. The HSP 27 ASO enhances apoptosis and delays prostate tumor progression (14), and chemosensitizes bladder, prostate, ovarian and uterine cancer to paclitaxel (14, 67, 68), colorectal cancer to irinotecan (69) and human gastric cancer cell lines resistant to vincristine (sgc7901/vcr) (70). Moreover, the HSP 27 ASO restores the apoptotic response to dexamethasone in a dexamethasone-resistant multiple melanoma cell line by triggering the release of the mitochondrial protein Smac, followed by activation of caspase-9 and caspase-3 (71). This abbreviated review illustrates that HSP 27 functions as a "hub" in multiple signaling and transcriptional pathways, identifying it as an attractive therapeutic target by simultaneously affecting many pathways implicated in cancer progression and resistance.

Interestingly, HSP 27 has been found to be increased during the early phase of stem cell differentiation, which is accompanied by a decreased rate of cellular proliferation, allowing differentiating cells to escape from apoptosis. Therefore, it is possible that targeting HSP 27 expression by siRNA, ASOs or small-molecule inhibitors might affect stem cell differentiation and survival (72). Moreover, it is very important to understand the role of cancer stem cells, progenitor cells and differentiated cells in treatment response, and this may change our thinking about cancer cell biology. Hence, the role of HSP 27 in prostate cancer stem cell growth, survival and response to treatment is currently unknown, but may be highly significant in future approaches to prostate cancer treatment.

PHASE I CLINICAL TRIALS WITH ANTISENSE OGX-427

A phase I clinical trial targeting HSP 27 with OGX-427 in breast, ovarian, prostate, bladder and non-small cell lung cancer was initiated in 2008. The primary objectives of the study were to define the pharmacokinetic and toxicity profile and determine a recommended phase II dose of OGX-427 given as a 2-hour i.v. infusion every week after three loading doses given over the first week. Secondary objectives were to evaluate antitumor activity by post-treatment tumor marker declines, measurable disease response and time to disease progression. Six patients per cohort were recruited at a starting dose of 200 mg i.v. weekly, with a planned maximum dose of 1000 mg to be evaluated. Patients with cancers known from the literature to express HSP 27 were included (breast, ovarian, prostate, bladder and non-small cell lung cancers). Doses of up to 1000 mg were administered and dose-limiting toxicity was not observed, with most adverse events being only grade 1 or 2 (mild to moderate) in severity. Related adverse events included infusion reactions, which included chills, pruritus and flushing. No patients were noted to have a partial response in measurable disease, although there were suggestions of single-agent anticancer activity: three patients had minor responses or stable disease for 3 months or more. Declines in tumor markers were also observed: 3 of 5 patients with ovarian cancer had decreases in the CA-125 tumor marker of 27-63% and 3 of 15 patients with prostate cancer had PSA declines of 42-77% from baseline. Serial enumeration of circulating tumor cells (CTCs) from patients' peripheral blood was performed using the Veridex immunomagnetic system. With this system, a decrease in CTC counts to 5 or less per sample has been associated with improved prognosis (2). In the trial, 24% of patients achieved this result, again suggesting single-agent anticancer activity. This phase I study also included patient cohorts that evaluated combination of

OGX-427 with docetaxel, and determined that both doses of 800 and 1000 mg i.v. weekly of OGX-427 were tolerable in combination with standard-dose docetaxel. A phase II randomized study of single-agent OGX-427 in patients with metastatic CRPC is set to commence in 2011.

CONCLUSION

HSP 27 is a multifunctional molecular chaperone that regulates the activity of many important survival signaling and transcriptional pathways known to be relevant in many advanced cancers, including CRPC. Overexpression of HSP 27 confers broad-spectrum treatment resistance and increased activity of AR (62), STAT3 (19), eIF4E (37), Akt and NF-κB (35) pathways. HSP 27 knockdown using ASO or siRNA induces apoptosis and chemosensitizes cancer cells to a wide variety of drugs. HSP 27 is frequently expressed in several types of cancer and has been associated with progression and poor prognosis. Thus, HSP 27 is an attractive therapeutic target for the treatment of cancer. OGX-427 is a second-generation ASO targeting HSP 27 currently undergoing clinical testing.

DISCLOSURES

The University of British Columbia has submitted patent applications listing Dr. Gleave as inventor on the antisense sequence described in this paper. This IP has been licensed to OncoGenex Pharmaceuticals, Inc., a Vancouver-based biotechnology company that Dr. Gleave has founding shares in.

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