Ann. Cancer Res. Therap. Vol. 15, No. 2, pp. 35-40, 2007

Hyperthermic sensitizers targeting heat-induced signal transductions

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Abstract

Hyperthermia has been used as one of the efficacious cancer therapies. To develop more efficient regimens for various malignant tumors, the attention has focused on searching for substances to sensitize cell lethality of tumors by hyperthermia. Inhibitors which interfere with anti-apoptosis and/or cellular proliferation signal transductions are good candidates for enhancers of heat sensitivity of cancer cells. This review summarizes signal transductions for anti-apoptosis and/or cellular proliferation after heating and inhibitors targeting key factors in these signaling transductions. The attractive sensitizers described here might contribute to high curative efficiency in hyperthermic cancer therapy.

Keywords: hyperthermic sensitivity, heat, apoptosis, inhibitor, sensitizer

(Received August 21, 2007; Accepted August 31, 2007)

Introduction

Recent studies have addressed that heat induces signal transduction pathways for apoptosis such as p53-centered[1] and c-Jun N-terminal kinase (JNK)-centered signal transduction pathways[2,3]. However, heat simultaneously activates signal transduction pathways for anti-apoptosis and/or cellular proliferation. Key signaling factors, such as Akt, p38, extracellular signal-regulated kinase (ERK) and heat shock protein (HSP), play important roles in anti-apoptosis/cellular proliferation pathway. Such signaling factors are negative for hyperthermic cancer therapy. Therefore, targeted inhibition of the signaling factors is an attractive therapeutic objective for the development of potent hyperthermic cancer therapies (Fig. 1). We previously summarized several inhibitors of the signaling factors[4]. In this review we newly report several inhibitors of the signaling factors.

1. Anti-apoptosis signal transduction induced by heat and inhibitors of signaling factors

1-1. Akt pathway

A serine/threonine kinase, Akt, plays important roles in anti-apoptotic/cell survival responses[5-7]. The activity of Akt is generally high in cancer cells, because amplification of the *Akt* gene is frequently observed in various types of cancer cells. Phosphatase and tensin homologue deleted on chromosome 10 (PTEN), which downregulates Akt activity, has been reported to be defective in various types of cancer cells. These abnormalities of Akt have been considered to be closely related to tumorigenesis. Therefore, Akt is now becoming a promising and attractive molecular target for enhancing apoptosis[8] and cancer therapy[9].

Akt is activated by heat through phosphatidylinositol-3-kinase (PI3-K) and the 3-phosphoinositide-dependent kinase-1 (PDK1)-mediated phosphorylation pathway[10]. The activity of Akt is maintained by HSP90 which protects Akt against dephosphorylation *via* protein phosphatase 2A (PP2A1)[11]. Activated Akt interferes with the heat-induced apoptosis pathway by phosphorylating caspase-9[12] and Bcl2-antagonist of cell death protein (Bad)[13,14]. Akt also blocks the translocation of orphan nuclear receptor HMR (Nur77) and Forkhead family proteins into the nucleus, resulting in the upregulation of Fas ligand and TNFRI-associated death domain (TRADD) protein expression as a result of phosphorylation of these transcription factors[12,13]. Furthermore, Akt phosphorylates nuclear factor κB (NF-κB). The phosphorylated NF-κB transcriptinally regulates the synthesis of proteins involved in cell survival[14] and thus it result in suppression of the efficiency of the cancer therapy[15]. The X-chromosome-linked inhibitor of apoptosis protein (XIAP) is one of the proteins regulated by NF-κB and is known to be an inhibitory factor for caspase-3. XIAP is involved in cell survival and plays a pivotal role in cancer progression[16]. High expression levels of XIAP are clinically associated with poor survival prognosis in patients[17]. Considering these facts, down-regulation of XIAP expression could be advanta-

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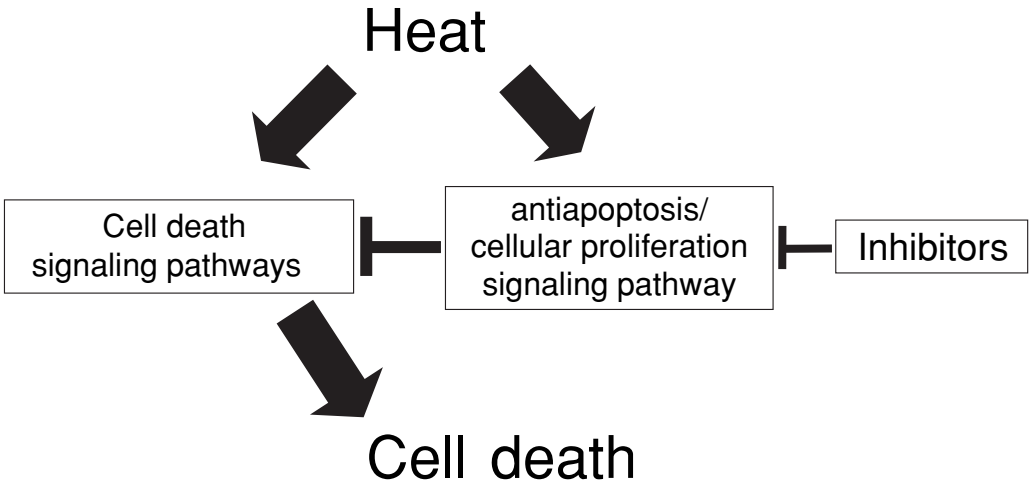

Fig. 1 A strategy for sensitization to heat sensitivity of cancer cells by inhibitors targeting heat-induced signal transduction pathways for anti-apoptosis/cellular proliferation

geous in cancer therapy. Heat-induced Akt activation is observed *in vivo* as well[18]. PI3-K activity increases after hyperthermia in the liver of rat *via* a tyrosine kinase-dependent mechanism[19]. Glycogen synthase kinase-3 (GSK-3), which is a possible downstream factor of PI3-K through Akt, undergoes hyperphosphorylation. Thus, *in vivo* heat-induced activation of the Akt pathway plays an important role in the protection against apoptosis[19]. Akt plays a central role in multiple pathways for the inhibition of heat-induced apoptosis, and thus one expects that interference with the Akt pathway by inhibitors would enhance heat-induced apoptosis.

1-2. Inhibitors of Akt pathway

1-2-1. PI3-K inhibitors

Wortmannin inhibits the activity of DNA-PK at lower doses and ATM at higher doses. As DNA-PK and ATM contribute to the DNA repair machinery, it has been reported that wortmannin enhances the radiosensitivity of cancer cells[20,21]. Another report showed that wortmannin enhances the radiosensitivity of ataxia telangiectasia cells, but it does not enhance the radiosensitivity of DNA-PK-deficient cells[22]. Therefore, it is possible that wortmannin sensitizes cells to radiation through inhibition of the DNA-PK-mediated DNA repair. Enhancement of heat-induced apoptosis by wortmannin has also been reported in a human breast cancer cell line[23]. 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002) sensitizes not only radiosensitivity but also heat-sensitivity[24]. The mechanism of the inhibition of heat-induced PI3-K activation seems to be different between wortmannin and LY294002. LY294002 p53-independently sensitizes cells to heat *via* the inhibition of heat-induced accumulations of survivin, hsp27 and hsp70[24]. Radio-sensitization with LY294002 has been reported by *in vitro*[20] and *in vivo*[25] experiments but heat sensitization with LY294002 has not been reported.

1-2-2. PDK1 inhibitor

7-hydroxystaurosporine (UCN-01) has been used as a PDK1-inhibitory drug in clinical trials. Unlike geldanamycin, UCN-01 induces Akt inactivation by inhibiting PDK1 directly, resulting in the suppression of the survival signals and the induction of apoptosis[26]. Staurosporine has also been reported to suppress PDK1 directly[26].

1-2-3. NF-κB inhibitor

Dicoumarol, a coumarin derivative, was reported to potentiate TNF-induced apoptosis in HeLa cells, probably by blocking the anti-apoptotic effect of NF-κB and is currently used clinically[27]. Since NF-κB is a target of Akt, dicoumarol is a potential hyperthermic cancer therapeutic inhibitor of NF-κB.

1-2-4. XIAP inhibitors

Enhancement of heat sensitivity of cancer cells by XIAP inhibitors has not been reported yet. However, enhancement of TRAIL-induced apoptosis, chemosensitivity or radiation sensitivity has been currently reported[28-31]. An XIAP inhibitor, embelin identified primarily from the Embelia ribes plant, enhances TRAIL-induced apoptosis *via* expression of XIAP in pancreatic cancer cells[28]. Embelin also blocks NF-κB signaling pathway leading to suppression of NF-κB-regulated anti-apoptosis[29]. Small interference RNA targeting XIAP (XIAP-siRNA) combined with paclitaxel, cisplatin, fluorouracil, and etoposide enhances chemosensitivity in esophageal carcinoma cells[30]. We recently showed that XIAP-siRNA enhances radiation sensitivity of lung can-

cer cells[31].

1-3. HSP inhibitors

HSP27, 72 and 90 play inhibitory roles in varied signal transduction pathways for apoptosis. One of these roles is to interfere with the formation of the apoptosome, which consists of apoptosis protease-activating factor-1 (Apaf-1), caspase-9 and cytochrome c. HSP27, 72 and 90 interfere with apoptosome formation in different manners and consequently suppress the activation of caspase-3. HSP27 binds to cytochrome c released from the mitochondrion and blocks the binding of cytochrome c to Apaf-1[32]. Another anti-apoptotic function of HSP27 is to regulate the activity of Akt[33]. HSP72 and HSP90 bind to Apaf-1 and depress the activation of caspase-9[34-36]. HSP72 suppresses heat-induced apoptosis by inactivating JNK[2,37-39] or by antagonizing apoptosis-inducing factor (AIF)[40]. HSP90 is a molecular chaperone whose association is required for the stability and function of signaling proteins that promote the growth and/or survival of cancer cells. Client proteins associated with HSP90 include Akt, breakpoint cluster region (Bcr)-Ableson tyrosine kinase (Abl), Raf-1, ErbB1/epidermal growth factor receptor (EGFR), ErbB2/Her2, mutated p53 and hypoxia-inducible factor 1α (HIF-1α)[41].

HSP90 inhibitors such as geldanamycin and radicicol are attractive anti-cancer agents[42]. Geldanamycin and radicicol indirectly down-regulate the activity of Akt through interfering with the association between HSP90 and PDK1. After the dissociation of PDK1 with HSP90, the PDK1 is proteasome-dependently degraded and the degradation of PDK1 results in elimination of the binding of PDK1 to Akt. The kinase domain of PDK1 is essential for complex formation with HSP90, and the inhibitors interact with this domain[43]. Geldanamycin and radicicol also alter the complex formed between HSP90 and Raf-1. This leads to a decrease in the Raf-1 level and consequently to disruption of the Raf-1-Map kinase-ERK kinase (MEK)-MAPK signaling pathway[44, 45].

17-allylamino-17-demethoxygeldanamycin (17-AAG) is a geldanamycin analog that is currently being used in Phase I clinical trials in the USA and UK[46]. 17-AAG also affects the Akt-mediated signal transduction pathway involved in tumor cell proliferation and survival. Early results from phase I trials have demonstrated that 17-AAG has an inhibitory function similar to that of geldanamycin but shows a significantly improved toxicity profile.

A coumarin antibiotic, Novobiocin, interacts with an ATP-binding domain in the carboxyl terminus of HSP90 and suppresses the chaperone function of HSP90[47]. Novobiocin is already being used in cancer therapy[48].

Quercetin and tamoxifen reduce heat shock protein-70 expression at both protein and mRNA levels and synergize with hyperthermia in reducing the clonogenic activity of melanoma and in inducing apoptotic cell death[49]. Quercetin and tamoxifen can be usefully combined with hyperthermia in recurrent and/or metastatic melanoma.

Specific inhibition of Hsp27 expression using an antisense oliogodeoxynucleotide increased the irinotecan sensitivity. Lower expression of Hsp27 kept caspase-3 activity in colorectal cancer cells[50]. From this, some kinds of an antisense oliogodeoxynucleotide against hsps could be candidates for heat sensitization of cancer cells.

A newly synthesized chemical, N-formyl-3, 4-methylenedioxy-γ-butyrolactam (KNK437) suppresses the induction of HSPs at the mRNA level. Since KNK437 does not affect the constitutive amounts of HSPs, the inhibitory mechanism of this compound seems to be due to inhibition of the activation of heat shock factor 1 (HSF1) or the binding of HSF1 to heat shock element (HSE). Based on this manner of inhibition, KNK437 is regarded as a potentially useful agent to suppress the heat tolerance of cancer cells which is frequently observed as a negative effect of fractionated hyperthermic cancer therapy[51-53].

2. Cellular proliferation signal transduction induced by heat and inhibitors of signaling factors

2-1. Mitogen-activated protein kinase (MAPK) cascade

The mitogen-activated protein kinase (MAPK) pathway is a key signal transduction cascade that links diverse extracellular stimuli to proliferation, differentiation, and survival[54]. Heat activates the MAPK cascade (ceramide to Ras/Raf/MEK/ERKs)[55-58] called the classical MAPK cascade. This cascade induces activation of intracellular substrates including transcription factors, such as Ets-like protein 1 (Elk-1), c-Jun, and activating transcription factor 2 (ATF2), and other protein kinases[59]. Inhibition of the activity of ERK1 by overexpression of a dominant-negative ERK1 enhanced the heat sensitivity of cells. In contrast, cells stably overexpressing the wild-type ERK1 developed resistance to killing by heat[55]. Ceramide activates Raf-1 *via* metabolism to sphingomyelin after heat shock. The activation of MAPKs by heat is cell type-specific, because myeloid leukemic cells such as HL-60, U937 and K562 cells have no ability to activate Raf-1, while NIH3T3 fibroblasts do possess such ability[55]. The activation of the MAPKs cascade is lacking in some types of cancer cells. MAP kinase kinases (termed MEK1 and MEK2) involved in downstream signaling of Raf-1 activate ERK1/2 by phosphorylation of both threonine and tyrosine residues. Heat shock induces ERK1/2 activation in rat brain[19]. Inhibition of the MAPKs cascade, in which a key target kinase is MEK, is expected to provide sensitization of cancer cells to hyperthermic cancer therapy.

2-2. Stress-activated MAPK, p38

p38, called stress-activated MAPK, has been characterized based on its activation in response to extracellular stress stimuli, including heat stress *in vitro*[60,61] and *in vivo*[19,20,62]. p38 is involved in a phosphorylation cascade (ceramide to MAP kinase-ERK kinase kinases (MEKKs)/apoptosis signal-regulating kinase1 (ASK1)/ MAP kinase kinases (MKKs)/p38) that is distinct from the above-described Ras/Raf/MEK/ERKs cascade[63], and it is involved in the regulation of cellular proliferation, differentiation and transformation[64,65]. *In vivo* experimental results have shown that MKK3 and/or MKK6 are activated downstream to MEKKs in response to hyperthermia in rats[20]. These reports taken together suggest that selective inhibition of p38 is also useful for sensitization to heat sensitivity. However, there are some reports showing that activation of p38 seems to be involved in the induction of apoptosis in some cell types upon various stress stimuli[66-68]. This discrepancy in the functions of p38 may result from the different genetic backgrounds among cancer cells.

2-3. Inhibitors of MAPK pathway

2-(2'-amino-3'-methoxyphenyl)-oxanaphthalen-4-one (PD98059) is an inhibitor that selectively depresses the activity of MEK. Inhibition of MEK by PD98059 prevents subsequent phosphorylation of ERK substrates that contribute to cell growth and survival[69,70]. PD98059 abrogates the clonogenicity of leukemic cells but has minimal effects on normal hematopoietic progenitors[71]. The suppressive function of PD98059 has been reported to be effective in transplanted tissue[72] or solid tumors[73]. PD98059 or U0126 enhances paclitaxel-induced apoptosis in solid tumor cell lines[73]. We have found that PD98059 sensitizes non-small lung cancer cells to heat *via* the inhibition of heat-induced accumulation of hsp27 and hsp70 and enhanced apoptosis through caspase-3 activation (Ohnishi et al., unpublished data).

4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole (SB203580) suppresses p38 activation selectively and consequently interferes with signaling induced by transforming growth factor-β(TGF-β)[74]. Inhibition of p38 by SB203580 induces enhanced heat sensitivity of lung cancer cells (Ohnishi et al., unpublished data) and suppresses invasion of cancers in which p38 is activated[75]. In contrast to the positive function of SB203580, a negative function that SB203580 leads cells to become resistant to cisplatin has been reported[76]. RWJ-67657[77] and FR167653[78] are also inhibitors of p38.

Acknowledgment

This work was supported by Grants-in-Aid from the Ministry of Education, Science, Sports, Technology and Culture of Japan.

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