

Cancers

Review

Targeting Heat Shock Protein 27 in Cancer: A Druggable Target for Cancer Treatment ?

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Abstract

Heat shock protein 27 (HSP27), induced by heat shock, environmental, and pathophysiological stressors, is a multi-dimensional protein that acts as a protein chaperone and an antioxidant. HSP27 plays a major role in the inhibition of apoptosis and actin cytoskeletal remodeling. HSP27 is upregulated in many cancers and is associated with poor prognosis, as well as treatment resistance whereby cells are protected from therapeutic agents that normally induce apoptosis. This review highlights the most recent findings and role of HSP27 in cancer, as well as strategies for using HSP27 inhibitors for therapeutic purposes.

Keywords: Heat shock protein 27; HSP27 inhibitor; Anti-cancer drugs, Resistance

1. Introduction

Heat shock protein (HSP) is a protein family produced in cells by stressors, such as hypoxia, hyperoxia, UV light exposure, viral agents, and nutritional deficiencies. Its key role is to maintain cellular homeostasis, promoting cell survival in lethal conditions. It associates with key regulatory proteins, such as transcriptional factors, protein kinases, and hormone receptors [1]. HSPs protect cells by acting as molecular chaperones which correct misfolded proteins. There are many types of HSPs and their functions vary slightly. Although there are a few ways to classify HSPs, one way is to classify them by their molecular weight. For example, the HSP27 molecule is 27kDa in size. Using their molecular weights, mammalian HSPs can be classified into six families: HSP100, HSP90, HSP70, HSP60, HSP40, and small HSPs (sHSP, 15 to 30kDa) [2].

HSP27 is a type of small HSP (sHSPs). The mammalian sHSP family, also known as the HSPB family, contains ten members: HSPB1 to HSPB10, HSP27/HSPB1, MKBP (Myotonic dystrophy protein kinase-binding protein)/HSPB2, HSPB3, α A-crystallin/HSPB4, α B-crystallin/HSPB5, HSP20/HSPB6, cvHSP (cardiovascular heat shock protein)/HSPB7, HSP22/HSPB8, HSPB9, and ODF1 (outer dense fiber protein)/HSPB10 [3]. HSP27 is encoded on the HSPB1 gene and belongs to a family of ATP-independent chaperones. HSP27 is a single copy gene covering 2.2kb transcripts organized in three exons encoding a 205 amino acid protein. The mechanism about its substrates and chaperone function have not been fully studied compared to other large HSPs. HSP27 is reported to be involved in cell resistance to stress factors and heat shock. However, the function of HSP27 is hijacked during disease and HSP27 helps to promote disease, rather than appropriately regulating cell homeostasis. HSP27 is present in both the cytoplasm and nucleus. It can be localized into the nucleus upon heat shock or exposure to various stress conditions. It was shown that overexpression of HSP27 promoted recovery from aggregation of heat-induced nuclear-protein [4], suggesting that HSP27 was partly responsible for consequent cell survival. Therefore, HSP27 plays a fundamental role in cell physiology in various disease status including cancer (Fig. 1).

In the following section, we present an overview of HSP27 and discuss the highly complex patterns of HSP27 phosphorylation and oligomerization in relation to its function. We also examine inhibitors targeted to HSP27 as cancer treatment strategies.

2. Structure of HSP27

Small HSPs are the most diverse in structure among the molecular chaperones. They are characterized by a sequence of about 100 amino acid residues, called an α -crystallin domain [4]. In humans, the α -crystallin domain plays an important role in dimer formation [5]. The structure of α -crystallin is dynamic and is affected by rapid subunit exchange under the stress conditions [6]. HSP27 acts as a chaperone to form multimeric complexes in cells and to stabilize denatured or aggregated proteins and return them to their original form [7]. Since the oligomeric form of HSP27 and the monomeric form occur dynamically, further research is needed to determine whether oligomers or monomers are actually required for protein homeostasis or what they do.

HSP27 has 250 amino acids and the sequence of human HSP27 is:

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1 mterrvpfsl lrgpswdpfr dwyphsrlfd qafglprlpe ewsqwlggss wpgyvrplpp
61 aaieSPavaa paysralsrq lssgvseirh tadrwrvsld vnhfapdelt vktkdgvvei
121 tdkheerqde hgyisrcftr kytlppgvdp tqvssslSPe gtlvveapmp klatqsneit
181 ipvtfesraq lggpeaaksd etaak
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X-ray analysis has revealed the crystallin domain of HSP27, however, the entire molecular structure is still unknown. Therefore, by using *Psipred*, a secondary structure helix and strand was deduced from the amino acid sequence of HSP27 (Fig. 2). Since the coil is too flexible, a complete tertiary structure has not yet obtained and may not be obtainable. HSP27 has a poorly conserved, disorganized N-terminal and a highly flexible, variable C-terminal. HSP27 is likely to change its structure depending on conditions, such as pH and temperature. It might be possible to obtain a tertiary structure in very specific conditions. HSP27 contains a poorly-conserved WDPF domain region, a highly-conserved α -crystallin domain region with β -sheets, a partially conserved PSRLFDQXFGEXLL sequence, and a flexible C-terminal. The WDPF domain name was derived from the amino acid residues it contains: W (tryptophan), D (aspartic acid), P (proline), and F (phenylalanine). The α -crystallin structure is important in oligomerization and solubility. HSP27 is an ATP-independent molecular chaperone involved in the protein folding-refolding machinery [8]. Several studies have shown that constitutively expressed HSP27 has apparently irrelevant cellular functions that can lead to interact with many other protein partners [9]. Therefore, it is crucial to understand the structure of HSP27 in order to grasp the structure-function relationship, including modulation of the activity and half-lives of many crucial client polypeptides [10]. A major challenge in HSP27 investigations is to determine the factors affecting its activity, including the level of oligomerization, the interaction with protein partners of

different molecular weights, etc., so that regulators of its activity, which can be potential anti-cancer treatments, can be developed. It is logical to assume that both the levels of oligomerization and the interaction with protein partners are involved but this is very difficult to determine with certainty because the ratio of HSP27 polypeptides interacting with the partners can be highly variable [11].

3. Oligomerization and phosphorylation of HSP27

HSP27 is phosphorylated in response to a variety of stressors. On the molecular level, HSP27 is phosphorylated by inflammatory cytokines, such as tumor necrosis factor- α (TNF- α); interleukin-1 β (IL-1 β); transforming growth factor beta (TGF- β); mitogens, such as insulin-like growth factor-1 (IGF-1); and steroid hormones. HSP27 is able to form oligomers up to 1000 kDa. α -Crystallin plays a key role in oligomerization as it forms a dimer, the molecular base of the oligomeric complex. In addition, a conserved tripeptide (I/V/L)-X-(I/V/L) motif on the C-terminal interacts with a hydrophobic groove on the surface of the core α -crystallin domain of a neighboring dimer. The dimer of HSP27 acts as the building block for multimeric complexes. Thus, it can control the structural plasticity of oligomeric sHSP.

The oligomerization of HSP27 is regulated by phosphorylation. When HSP27 is not phosphorylated, it forms an oligomer. Electronic microscopy and X-ray crystallography images indicate that oligomers form ring-like structures with symmetrically packed dimers inside. Since α B-crystallin subunits cannot interact with unfolded proteins, phosphorylated HSP27 shows decreased chaperone activity. Therefore, oligomerization and phosphorylation direct the biological activity and function of HSP27.

Human HSP27 can be phosphorylated on three serine residues (Ser15, Ser 78, and Ser82) and on threonine (Thr143) by multiple kinases, including MAP kinase-activated protein kinase 2 (MAPKAPK2) and ribosomal S6 kinase (p90RSK), protein kinase C (PKC), PKD, and PKG [12,13]

The contribution of HSP27 to single phosphorylation in one of these sites in the biological process has not been studied yet. However, previous studies have reported that Ser78 and/ or Ser82 contribute significantly to the oligomerization of HSP27, but that Ser15 has only minor effects. [12,13]. HSP27 phosphorylation/dephosphorylation equilibrium has been shown to be regulated by kinase and phosphatase (Fig. 3). Phosphorylation favors the formation of small oligomers, while dephosphorylation favors the formation of large oligomers and is a reversible event that regulates the oligomerization of protein [12,13]. However, recent studies have shown

that phosphorylation of HSP27 reduces its oligomerization ability *in vivo* cell-based assays, while *in vivo* oligomerization is linked to cell-cell contact and is independent of phosphorylation status [14]. HSP27 can form oligomers up to 1,000 kDa, which is a very dynamic process that plays a central role in modulating the chaperone activity of HSP27, a competent, binding state of the client protein [15] (Fig. 3). According to recent studies, the dimeric form of HSP27 is central to enhanced chaperone activity, demonstrated by increased binding to other client proteins [16,17].

The alpha-crystallin domain of murine (C141) and human (C137) HSP27 [9] and that on the beta-7 strand of several human other HSPs has been proposed to play a pivotal role in the inter-subunit contact of several human sHSPs [18]. Deletion or mutation of the unique cysteine blocks dimer formation, which consequently alters multimer formation, suggesting cysteine residue of HSP27 is important for its chaperone activity and its ability to interact with many polypeptides [19].

4. The role of HSP27 in cancer

Overexpression of HSP27 are closely related to tumorigenesis, metastasis, and invasiveness, and thus, to poor prognosis in various cancers [20,21]. Increased expression of HSP27 is also found to be associated with resistance to chemotherapy drugs in cancer cells [22]. The cytoprotective function of HSP27 is associated with chaperone functions, direct interference with apoptosis pathway, the promotion of drug resistance, and the regulation of cytoskeleton dynamics [23]. HSP27 has been shown to protect cells from death signals induced by different ways, including apoptosis, necrosis, and various physiological stresses [24,25]. HSP27 inhibits both intrinsic and extrinsic apoptotic pathways through binding of its small or large oligomeric form to cytochrome C or DAXX, respectively [26,27]. HSP27 inhibits caspase 9, depending on the activity of Bcl-2-associated X protein (BAX), which is activated by the pro-apoptotic protein BID. HSP27 also interacts with Protein kinase C delta type (PKC δ) and induces resistance to cancer therapy [28]. Moreover, the interaction between HSP27 and I κ B is involved in the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B) [29]. It can interact with microtubule actin protein, which is important for maintaining the integrity of the cytoskeleton and may help to promote cell survival and invasion [30] (Fig. 4).

Antisense oligonucleotides and small interference RNA (siRNA) to HSP27 increases apoptotic rates and enhances chemotherapy activity [1]. HSP27 is highly expressed in anti-cancer drug-resistant cancers. Studies have reported increased levels of HSP27 in various types of cancer, such

as liver, breast, colorectal, melanoma, prostate, glioma, lung, gastric, rectal, pancreatic and kidney (Table 1). Therefore, HSP27 can be an important therapeutic target, especially in cancer because it plays a major role in cell apoptosis or multiple cellular pathway under stress conditions in cells.

In cancer patients, the overexpression of HSP27 is associated with poor prognosis for patients and HSP27 become an object of research investigating factors involved in the invasion and metastasis affecting key factors for overall survival of patients. Recent studies analyzed the HSP27 levels in serum and in tumor microenvironments and the serum HSP27 levels were significantly higher in patients with prostate and breast cancer than in the control group [31]. Moreover, HSP27 levels was also reported to be related to the overall survival of patients with many other types of cancer, such as gastric, lung, liver, breast, kidney and rectum adenocarcinoma (Fig. 5).

Recent clinical trials have investigated the inhibition of HSP27 can be a molecular target for cancer therapy. However, unlike other HSPs, which bind ATP, HSP27 is an ATP-independent chaperone and this makes targeting HSP27 with the small compounds difficult. However, a recent study suggested a novel strategy to inhibit HSP27 by inducing cross-linking of HSP27 proteins. By inserting between the disulfide bond of HSP27, the cross-linking of HSP27 was altered and the normal HSP27 dimerization was disrupted, which resulted in an inhibition of functional HSP27.

Moreover, the altered dimerization of HSP27 can sensitize cancer cells with high HSP27 expression [32]. This strategy is expected to overcome drug development currently limited by the absence of HSP27 inhibitors and presents the possibility of the development of novel HSP27 inhibitors (Fig. 6).

5. HSP27 inhibitors for cancer treatment

5.1. Small molecules

5.1.1. RP101 (Brivudine)

RP101 (known as BVDU, bromovinyldeoxyuridine, brivudine) is a nucleoside that can inhibit HSP27 function via binding π -stacking with Phe29 and Phe33 of HSP27. When RP101 binds to HSP27, the binding of HSP27 to Akt1, pro-caspase3, and cytochrome C is weakened and affects apoptosis. Therefore, RP101 functions as a chemo-sensitizing agent to anti-cancer drugs. In vitro experiments showed that mitomycin C (MMC) with RP101 inhibited cell growth after heat shock [33]. RP101 inhibited the resistance of rat sarcoma cells to MMC by reducing their growth by 5 folds compared to the MMC alone group [34]. RP101 combined with gemcitabine in

fibrosarcoma cells reduced invasiveness by 30-50% compared to gemcitabine alone. RP101 with cisplatin or cyclophosphamide significantly inhibited the tumor growth of AH13r sarcomas-grafted SD-rats [34]. These data suggest that RP101 is more effective with combination treatment such as cytotoxic drug than mono-therapy. In clinical studies, RP101 increased the overall survival rate of patients with pancreatic cancer by 8.5 months compared with the control group [34]. Phase II clinical trials for the treatment of pancreatic cancer using gemcitabine with RP101 increased the median survival of approximately 2.17 months. However, overuse of RP101 showed the increased toxic side effects of gemcitabine in some patients [34], indicating the limitation of RP101 in clinical application.

5.1.2. Quercetin

Quercetin, a bioflavonoid widely distributed in plants, is well known as one of the natural compounds with anticancer properties [35]. Quercetin suppresses the heat shock transcriptional factor1 (HSF1) dependent induction of the HSPs [36,37], and shows anti-tumor effects in oral, hepatoma, prostate cancer, glioblastoma, squamous, gastric, breast cell lines and various cancer stem cells [38-43]. In lung cancer cells (A549), cisplatin or gemcitabine against A549 cells inhibits cell viability with quercetin compare to alone [44]. Quercetin acts as a chemo-sensitizer when used with first line chemotherapeutic drugs such as 5-fluorouracil, gemcitabine, doxorubicin and cisplatin. However, quercetin has not been identified in the exact mechanism, and further studies are needed to directly inhibit HSP27.

5.1.3. Altered dimerization of HSP27 using small molecules

According to recent studies, zerumbone [45], isolated from a natural product, and SW15, a synthetic xanthone compound, induced cross-linking of HSP27 protein by inserting disulfide bonds between HSP27 [46]. The same xanthone moiety with different side chains caused different cross-linking activity of HSP27. The HSP27 Cys residue is important for the altered cross-linking of HSP27 by the xanthone compound. The combination of anti-cancer drugs and the xanthone compound sensitized NSCLC cells. The Cys residue of HSP27 is important for sensitization of cancer cells by the xanthone compound in combination with anti-cancer drugs. Also, the xanthone compound sensitized cancer cells in combination with radiation. J2, a synthetic chromone compound, has a pharmacophore structure and more potent cross-linking activity than SW15 [32]. Therefore, the alteration of cross-linking is considered to be a novel strategy for the

inhibition of HSP27-mediated resistance in lung cancer (Fig. 7).

5.2. Antisense drug

A second-generation antisense oligonucleotide (ASO) targeting mRNA of HSP27, OGX-427 (OncoGeneX Pharmaceuticals), decrease the expression of HSP27. In prostate cancer xenograft, OGX-427 with chloroquine decreases tumor volume compare to chloroquine alone [47]. Xenograft of pancreatic and lung cancer also showed that when combined with gemcitabine, erlotinib and OGX-427, decrease tumor size compare to alone [47-49]. In the phase I clinical study, response occurred in 33% of 15 metastatic bladder cancer patients. In the phase II on castrate resistant prostate cancer patients, 71% of patients showed progression-free at 12 weeks when OGX-427 was combined with prednisone [50].

Inhibition of HSP27 significantly increased radiation-induced apoptosis and clonogenic death and promoted Akt inactivation. HSP27 knockdown improved the efficacy of radiation therapy by improving the cytotoxic effects of radiation therapy in patients with radiation resistant lung cancers [51]. The combination of OGX-427 and local tumor irradiation resulted in significant regression in SQ20B cancer cells-bearing mice and a decrease in glutathione antioxidant defenses and cell survival [51].

Tumor therapy with radiation to OGX-427 resulted in decreased angiogenesis associated with decreased activation of the Akt pathway. The combination therapy improved the survival and anti-cancer effect of Sq20B cancer cells-bearing mice and did not show signs of acute or delayed toxicity [51]. However, clinical study reported that the treatment of OGX-427 to a standard chemotherapy regimen, did not result in increased survival in unselected patients with metastatic pancreatic cancer, but there was a trend toward prolonged progression-free survival and overall survival in patients with high baseline serum HSP27, suggesting that this therapy may warrant further evaluation in the subgroup [52].

5.3. Peptide aptamers

The use of specific peptides to inhibit the anti-apoptotic activity of HSP27 has become a new approach to chemotherapy because of the difficulty of dealing with antisense technology in vivo. Protein aptamers are small amino acid sequences that are inserted into a scaffold protein. They bind to specific protein domains and are designed to regulate the activity of various cellular proteins, including oncogenes, transcription factors, cell cycle regulators, and others [53]. Recent

research showed that peptide aptamers could interact with HSP27 and promote the apoptosis of cancer cells. PA11 and PA50 specifically bind to HSP27, interfering with dimerization and oligomerization of HSP27, and could act as negative regulators of HSP27 functions. PA11 prevents HSP27 oligomerization, which finally results in the inability of HSP27 to inhibit cellular proteostasis. PA50 primarily inhibits HSP27 dimerization, disrupting the essential processes of cell survival by destroying the ability of HSP27 to participate in cell signaling events. These peptide aptamers also showed anti-tumor effects in mouse xenograft models [53]. Similar to the small molecule inhibitors of HSP27, peptide aptamer has a powerful effect when used with other anti-cancer drugs than alone.

Even though the pre-clinical success of peptide aptamers suggests the potential application to cancer therapy, there are limitations to the use of protein aptamers, including restrictions in the size of the investigated protein, the inability to deal with protein complexes and membrane components, as well as the difficulties of working in an RNase-free environment. Once these limitations are overcome, protein aptamers can be used to specifically target HSP27 to explore new insights into the HSP27 structure-function relationship and discover novel anticancer drugs.

6. Conclusion

In this review, we have discussed the role of HSP27 with respect to normal and stress conditions, especially cancer, with regard to the interaction of small heat shock proteins with other cellular molecules in various cellular processes. The structure of HSP27 differs greatly in other species, but functional forms such as oligomeric and dimeric forms of HSP27 are essential. Therefore, there is a need for research on diseases related to HSP27 and development of drugs in the future. HSP27 is an effective target for cancer treatment. Thus, the structural complexity of HSP27 challenges the discovery of therapeutic inhibitors that can neutralize HSP27. Moreover, recent studies have suggested the potential of inhibiting HSP27 as a therapeutic target for cancer. However, it is believed that, unlike other heat shock proteins, the small heat shock proteins, such as HSP27, lack an ATP binding site and this makes it difficult to think of HSP27 as an easy target for small molecule. It is obvious that researchers should focus their efforts in this direction to investigate potential new cancer therapies. In this sense, small molecules of cross-linking HSP27 may be promising for functionally inhibiting HSP27.

In conclusion, HSP27 is overexpressed in many cancers and associated with the development of resistance against anti-cancer drugs. Therefore, inhibitors of HSP27 may improve cancer

chemotherapy when used in combination therapy together with anti-cancer drugs.

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Figure Legends

Figure. 1. Major roles of HSP27

HSP27 has important functions in regulating protein folding, aging, cancer, the immune response, and the development and suppression of cellular apoptosis.

Figure. 2. The structure of HSP27.

The structure of human HSP27 consists of the N-terminal domain, the alpha-crystallin domain, and the C-terminal domain. The N-terminal domain contains a WDPF motif which is essential for large oligomerization. The C-terminal domain includes an alpha-crystallin motif that is highly conserved between species and is involved in the formation of small oligomerization. HSP27 can be phosphorylated by MAP kinase-activated protein kinase 2 (MK2).

Figure. 3. Conformational structural switching between different states.

HSP27 exists as large oligomers when unphosphorylated. At specific serine residues in the mitogen-activated protein kinase (MAPK) pathway, HSP27 switches to smaller oligomers. HSP27 conformational structure changes actively and contributes to maintaining proteostasis.

Figure. 4. Role of HSP27 in different cellular apoptotic processes.

HSP27 inhibits apoptosis by integrating different signaling pathways, including the extrinsic and intrinsic apoptosis pathway.

Figure. 5. Kaplan-Meier (KM) curves for HSPB1 (gene name of HSP27) in the overall survival of various cancers.

Gastric cancer, lung cancer, liver hepatocellular carcinoma, breast cancer, kidney renal clear cell carcinoma and rectum adenocarcinoma show high survival rates associated with low expression of HSPB1. P-values were calculated using the log-rank test. HR (Hazard Ratio) is the ratio of the hazard rates corresponding to the conditions described by two levels of an explanatory variable (HR>1 were considered higher hazard of death from the HSPB1 High group).

Figure. 6. Strategies of HSP27 inhibition

A) Three small molecule inhibitors. B) Peptide aptamers bind directly to HSP27 protein and inhibit oligomerization. C) Antisense oligonucleotide binds to HSP27 mRNA and prevents the expression of HSP27 protein.

Figure. 7. Altered cross-linking of HSP27 using small molecules for HSP27 inhibition. Scheme for the mechanism of HSP27-cross-linking by small molecules. Normal dimerization of HSP27 contributes to cancer cell survival but abnormal dimerization of HSP27 using small molecules causes cancer cell death.

Table 1. Functions of HSP27 in various cancer cells (year 2010~)

Cancers	Effects	References
Liver	Promotes proliferation and invasion of hepatocellular carcinoma cells.	[54,55]
Breast	Downregulation of HSP27 induces chemo-sensitization to Herceptin and inhibition of cancer cell proliferation. HSP27 regulates the EMT process and NFkB activity to contribute to the maintenance of BCSCs. Expression of phosphorylated forms of the chaperone HSPB1 correlates with the amount and percentage of lymph node metastases. Down-regulation of HSP27 in human breast cancer cells modulates down-regulation of PTEN.	[56-59]
Colorectal	Suppression of HSP27 protein expression enhances 5-FU sensitivity. Patients with low HSP27 expression shows better survival than those with high HSP27 expression. Acquired drug resistance of 5-FU is caused by the enhanced constitutive expression of HSPB1 and its phosphorylated form in colorectal cancer cells.	[60-62]
Melanoma	HSP27 expression is associated with impaired prognosis in melanoma. HSP27 is important for tumor dormancy, angiogenesis regulation, and tumor progress in cutaneous melanoma.	[63,64]
Prostate	HSP27 increases PCa cell motility, growth, and survival. Downregulation of HSP27 radiosensitizes human prostate cancer cells. In patients with prostate cancer, with HSP27 and Twist expression each elevated in high-grade prostate cancer tumors. DNA methylation of HSPB1 resulted in poor outcome in prostate cancer patients.	[21,65-67]
Glioma	Promotes glioma cell proliferation. Quantitative proteomic analysis shows that HSP27 is involved in the poor prognosis of GBN.	[68,69]
Lung	HSP27 inhibitor induces chemo-sensitization to anti-cancer drugs. Increased HSP27 expression correlates with shorter survival of NSCLC patients.	[32,70,71]
Gastric	Meta-analysis of gastric cancer is strongly dependent on the overexpression of HSP27.	[72,73]
Rectal	High expression of HSP27 represents poor survival in rectal cancer.	[74]
Pancreatic	Downregulation of HSP27 sensitizes to gemcitabine in gastric cancer cell line by regulating expression of Snail. HSP27 phosphorylation status contributes to gemcitabine resistance.	[75,76]
Kidney	Abnormal HSP27 phosphorylation is observed in renal	[77-79]

cancers, as well as in other kidney diseases.

In ccRCC patients, high serum HSP27 is associated with high grade (Grade 3-4) tumors.

TGF- β 1/p38/HSP27 signaling pathway inhibits cancer invasion and metastasis in RCC.

EMT, Epithelial–mesenchymal transition; BCSC, Breast cancer stem cell; NFkB, Nuclear factor kappa-light-chain-enhancer of activated B cells; 5-FU, Fluorouracil; PCa, Prostate cancer; NSCLC, Non-small cell lung cancer; GBM, Glioblastoma; ccRCC, clear cell renal cell carcinoma; RCC, Renal cell carcinoma

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