ROLE OF HEAT SHOCK PROTEINS IN CANCER TREATMENT

A PROJECT REPORT

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BONAFIDE CERTIFICATE

Certified that this project report titled **"ROLE OF HEAT SHOCK PROTEINS IN CANCER TREATMENT "** is the bonafide work of " **AMITASHA MISHRA(21BOE10018)**, **SHREYA DANDWATE(21BOE10068), SANAVI TAMBE(21BOE10052), DEVIKA RAJENDRAN(21BOE10016), VIKAS SINGH(21BOE10023), SOURABH TILLU(21BOE10035)"** who carried out the project work under my supervision. Certified further that to the best of my knowledge the work reported at this time does not form part of any other project/research work based on which a degree or award was conferred on an earlier occasion on this or any other candidate.

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ABSTRACT

This report explores the role of heat shock proteins (HSPs) in cancer treatment. HSPs are a family of proteins that play a critical role in protecting cells from stress and maintaining their normal function. However, HSPs are also overexpressed in many types of cancer and can promote tumour growth and metastasis. The report examines how HSPs contribute to cancer development and progression, and how targeting HSPs may be a promising approach to cancer treatment. It also discusses various strategies for inhibiting HSPs, such as small molecule inhibitors and immunotherapies. The report concludes that targeting HSPs has the potential to be a promising avenue for cancer therapy, and ongoing research in this area holds great promise for improving cancer treatment in the future.

CHAPTER 1
PROJECT DESCRIPTION AND OUTLINE

1.1 Introduction

Cancer is becoming one of the top causes of mortality on a global scale. Cancer is the biggest cause of mortality worldwide, according to the World Health Organization (WHO), accounting for an estimated 10 million deaths in 2020.According to the International Organization for Research on Cancer, the number of new cancer cases is predicted to climb by roughly 70% during the next two decades (IARC).

This report talks about a potential breakthrough in cancer treatment using heat shock proteins or HSPs. Cancer is a complex disease that affects millions of people worldwide, and its treatment can often be challenging and expensive. However, recent research has shown that HSPs could play a crucial role in cancer treatment.

In this report, we will first discuss what HSPs are and how they function within the human body. We will then explore the current methods of cancer treatment and the limitations of those methods. Next, we will delve into the research on using HSPs as a cancer treatment and the potential benefits of this approach. We will also discuss the challenges and potential risks associated with using HSPs for cancer treatment.

Heat shock proteins (HSPs) have been investigated as a possible cancer treatment due to their capacity to trigger immune responses against cancer cells and improve the efficacy of chemotherapy and radiation therapy.

According to current research, HSPs can be employed as therapeutic vaccines to boost the immune system and enhance cancer treatment outcomes. Numerous clinical trials have been carried out to assess the safety and efficacy of HSP-based cancer vaccines, with some encouraging findings.

A series of molecular steps occur to facilitate the transition of a normal cell into a cancer cell. Viruses, radiation, carcinogenic chemicals, and hereditary and non-hereditary genetic changes have all been identified as etiological agents and factors involved for commencing the carcinogenic process. Hence, we want to know how these cancer-causing chemicals might alter the molecular environment of altered cells, specifically the involvement of the Hsp.

Understanding the implications of such alterations is paving the way for cancer prevention, detection, and treatment options. Adenovirus, HPV, HBV, and HCV are among the oncogenic viruses that have been found; all of them have been linked to changes in the expression of certain Proteins.

Several of the roles attributed to heat shock proteins (Hsp) have been discovered via research into their role in tumour cell biology. Hsp90's chaperone function, for example, is vital for the maintenance of several client proteins required for cancer cell growth and survival. This has resulted in the investigation of a wide range of Hsp90 inhibitors, which interfere with the chaperone function of this protein, causing the destruction of mutated proteins and oncoproteins, as well as altering the stabilisations of "normal" proteins important for tumour progression, such as hypoxia-inducible factor 1, via ubiquitination and proteasomal degradation.

Understanding the consequences of such changes are paving the way to cancer prevention, diagnosis and treatment strategies. Among the identified oncogenic viruses are adenovirus, HPV, HBV and HCV; all of them have been related with changes in the expression of certain Hsp . Consequences of such alterations are cytoskeleton modifications characteristic such as seen in HPV infection. The cytoskeleton is very sensitive to different stressors, and the Hsp contribute to cytoskeleton organization .

1.2 Motivation of the work

One of the major challenges in cancer treatment is developing therapies that specifically target cancer cells while leaving healthy cells unharmed. HSPs have the potential to be an effective target for cancer therapy because they are overexpressed in many cancer cells and are critical for the survival and growth of these cells. Inhibiting HSPs could therefore lead to selective cancer cell death while leaving normal cells unharmed.

Another motivation for exploring the role of HSPs in cancer treatment is that they are involved in resistance to chemotherapy and radiation therapy. Cancer cells can develop resistance to these treatments by upregulating HSPs, which protect them from the damaging effects of these therapies. Targeting HSPs could therefore be a strategy to overcome resistance and improve the efficacy of these treatments.

In summary, the role of HSPs in cancer presents an important opportunity for developing new, targeted cancer therapies. Understanding the mechanisms by which HSPs regulate cancer cell growth and survival can lead to the development of novel treatments that specifically target cancer cells while sparing normal cells, and overcoming resistance to current therapies.

1.3 Problem Statement

Heat shock proteins (HSPs) are a class of molecular chaperones that play a critical role in maintaining cellular protein homeostasis. In addition to their role in normal cellular function, HSPs have been found to be upregulated in many cancer cells, where they contribute to tumor growth, survival, and resistance to therapy.

Recent studies have shown that HSPs can be potential targets for cancer treatment. Inhibiting the function of HSPs can lead to the degradation of oncoproteins and cause cancer cell death. Additionally, HSPs have been shown to be involved in the presentation of cancer antigens, making them attractive targets for cancer immunotherapy.

However, targeting HSPs for cancer treatment is not without its challenges. While inhibition of HSPs can be effective in killing cancer cells, it may also impact the function of normal cells. Additionally, some cancer cells may develop resistance to HSP inhibitors over time.

Overall, the role of HSPs in cancer treatment is an active area of research and holds promise for the development of new and effective cancer therapies.

1.4 Objective

Cancer is the world's largest cause of morbidity and mortality, accounting for roughly 10 million fatalities in 2020. Worldwide, an estimated 28.4 million new cancer cases (including NMSC, except basal cell carcinoma) are projected to occur in 2040, a 47% increase from the corresponding 19.3 million cases in 2020, assuming that national rates estimated in 2020 remain constant.

In terms of new cancer cases in 2020, the most prevalent were:

- breast (2.26 million cases);
- lung (2.21 million cases);
- colon and rectum (1.93 million cases);
- prostate (1.41 million cases);
- skin (non-melanoma) (1.20 million cases); and
- stomach (1.09 million cases).

However, The most common causes of cancer death in 2020 were:

- lung (1.80 million deaths);
- colon and rectum (916 000 deaths);
- liver (830 000 deaths);
- stomach (769 000 deaths); and
- breast (685 000 deaths).

Each year, approximately 400 000 children develop cancer. The most common cancers vary between countries. Cervical cancer is the most common in 23 countries.

Tobacco use, a high Body mass index (BMI), alcohol use, a lack of fruits and vegetables, and a lack of physical activity account for almost one-third of cancer fatalities.

Infections that cause cancer, such as human papillomavirus (HPV) and hepatitis, account for around 30% of cancer cases in low- and lower-middle-income nations.

Other risk factors can include genetics, exposure to certain chemicals, radiation exposure, and infections.

Many tumours can be cured if diagnosed early and treated appropriately.

Cancer may have a devastating effect on individuals and their families. Cancer can cause physical discomfort, exhaustion, and other symptoms that impair a person's ability to work, socialize and carry out everyday tasks. Cancer therapies such as surgery, chemotherapy, and radiation therapy can all have unpleasant side effects.

Furthermore, cancer can have a large influence on society as a whole. Cancer treatment expenditures and missed productivity can harm the economy, and the emotional toll of cancer can damage the mental health and well-being of entire communities.

Given that cancer cells frequently create large amounts of HSPs to aid in their survival under challenging conditions, there has been a great deal of interest in the possible application of HSPs in cancer treatment. One technique being investigated is to target HSPs in cancer cells with small molecules or other treatments that can inhibit their activity.

Hsp90, which is involved in the folding and stability of numerous proteins critical for cancer cell survival and proliferation, has earned special attention in cancer treatment. Several small molecule Hsp90 inhibitors have been created and are being investigated in clinical studies for the treatment of different cancers.

To conclude, cancer is a complicated and destructive disease that affects millions of individuals throughout the world. More research is required to better understand cancer's origins and to create more effective therapies. Cancer education and awareness can help decrease the disease's impact on people and society as a whole. Furthermore, more assistance

and resources are required to assist individuals and families impacted by cancer in coping with the disease's physical, emotional, and financial burdens.

CHAPTER 2

HYPOTHESIS

2.1 Hsp90

Introduction and Basic Processes

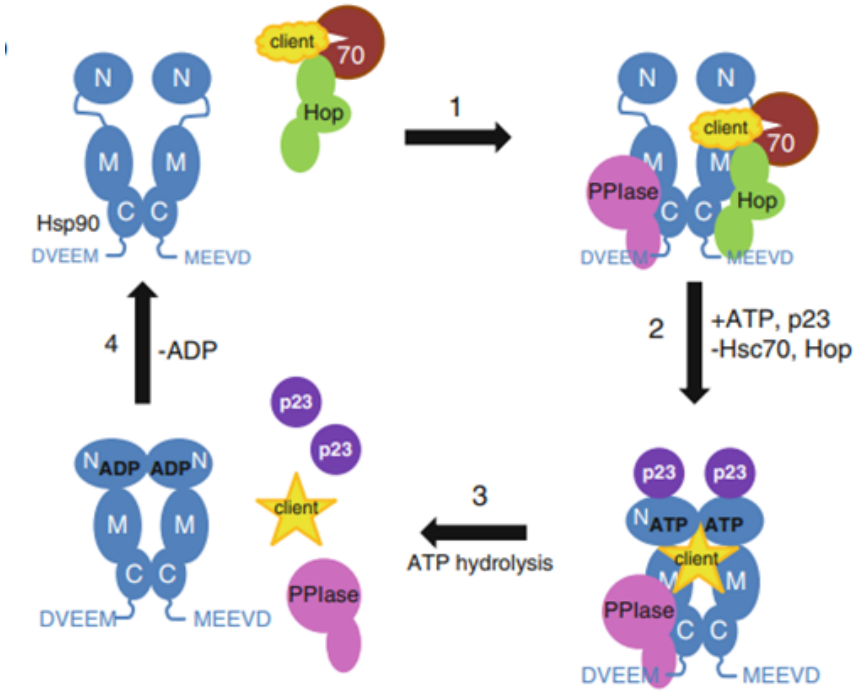
Hsp90 is a highly abundant and ubiquitous molecular chaperone that is found in all animal kingdoms except archaea which plays an essential role in cellular processes including cell cycle control, cell survival, hormone and other signaling pathways. It is important for the cell's response to stress and a key player in maintaining cellular homeostasis. Recently it is being used as a major therapeutic drug for cancer and neurodegenerative diseases also in antiviral and anti-protozoan infections.

Hsp90 is required for the correct maturation and activation of many cellular proteins which are collectively called "clients". The number of potential clients has increased dramatically in recent years.

Many Hsp90 clients are either kinases or transcription factors, including the kinases Akt, cdk4/6, B-Raf, Plk 1, src, and other tyrosine kinases, and steroid receptors, heat shock factor 1 (HSF 1), p53, v-erb A to name a few. Other client proteins are from structurally and functionally very diverse families – some bring ribosomal proteins, some being viral proteins and coat proteins or telomerase of NLR protein from plants, also certain proteins that are associated with neurodegenerative disorders.

How Hsp90 recognises this variety of different clients is a mystery but given the number of these client proteins that are either up-regulated or mutated in cancer Hsp90 is now a target for many therapeutic drugs against cancer.

A genome-wide study has also suggested that up to 10% of all proteins required Hsp90 for their function although many are in indirect processes. Hsp90 does not act alone but has a host of co-chaperones which regulate its activity and direct specificity on the machinery. A long-standing model for how Hsp90 and cochaperones cooperate in the folding and maturation of steroid receptors is shown in the figure below

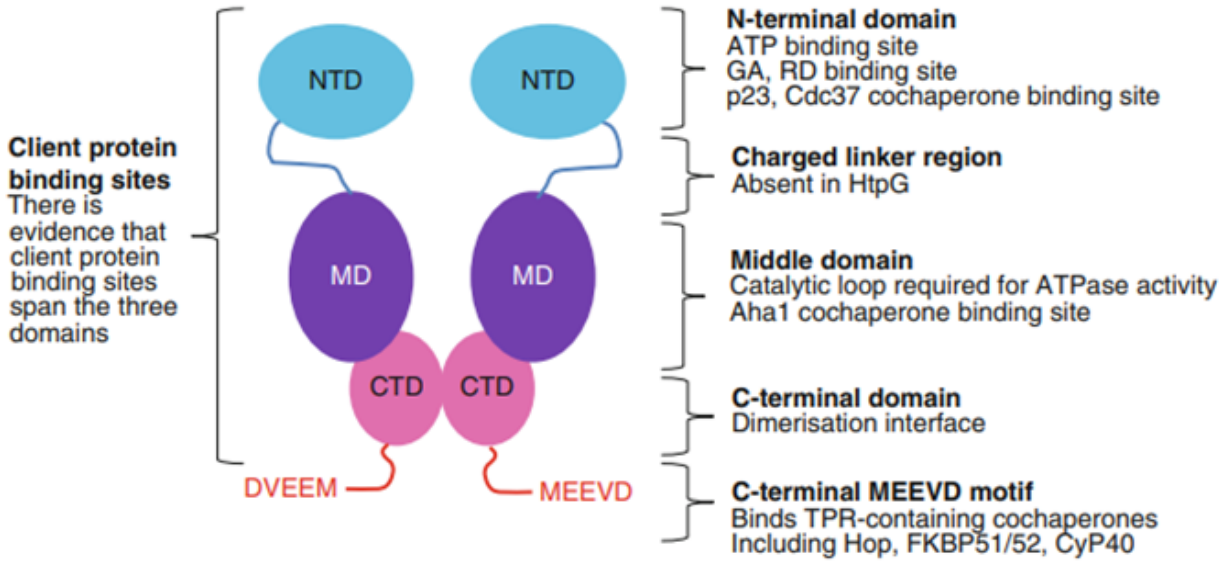


In this model, the newly synthesized receptor first interacts with the Hsp40/70 chaperones and then the receptor is efficiently transferred to Hsp90 by the action of Hop which can simultaneously bind to both Hsp70 and Hsp90. At this stage, Hsp90 is considered to have no nucleotides and is in an open conformation. The next step is the bonding of ATP, the closure of the structure and the dimerization of the N-terminal domains of Hsp90, the release of Hop and/or the Hsp70 machinery followed by the binding of other cochaperones such as p23, FKBP51/52 etc. The binding of an Hsp90 inhibitor like geldanamycin (GA), which binds to the ATP-binding site and prevents nucleotide binding, results in the blocking of the cycle and subsequent degradation of the receptor.

The biological activity and function of Hsp90 are crucially dependent on both its ATPase activity and its conformational dynamics including both structural changes within a single domain and changes in the conformation of Hsp90's 3 domains relative to each other.

Molecular Structure of Hsp90

Hsp90 has three structural domains- an N-terminal domain (NTD) which contains the ATP binding site, which is connected to a middle domain (MD) through a visibly charged linker, and a C-terminal domain (CTD) which is responsible for Hsp90 being a dimer. A visual representation is in the figure below.



The first part of Hsp90 to be discovered was the N-terminal domain which helped understand the activity of ATPase as the structures of the NTDs with its nucleotide bound confirmed that Hsp90 is an ATP binding protein and therefore has ATPase activity, this has been unconfirmed before as the basal ATPase activity is very low. This allowed the identification of the key catalytic residues and thus subsequent studies confirmed the ATPase activity.

The next part of Hsp90 to be discovered was the middle domain. It revealed a hydrophobic patch having an exposed tryptophan which is important for client protein binding. This domain also contains an important catalytic loop with a highly conserved Arg residue which is essential for the ATPase activity.

The C-terminal domain was the last part of the Hsp90 structure to be discovered. CTD is a homodimer of two small mixed α/β domains where the dimer interface is formed with the two α- helices which pack together to form a four-helical bundle.

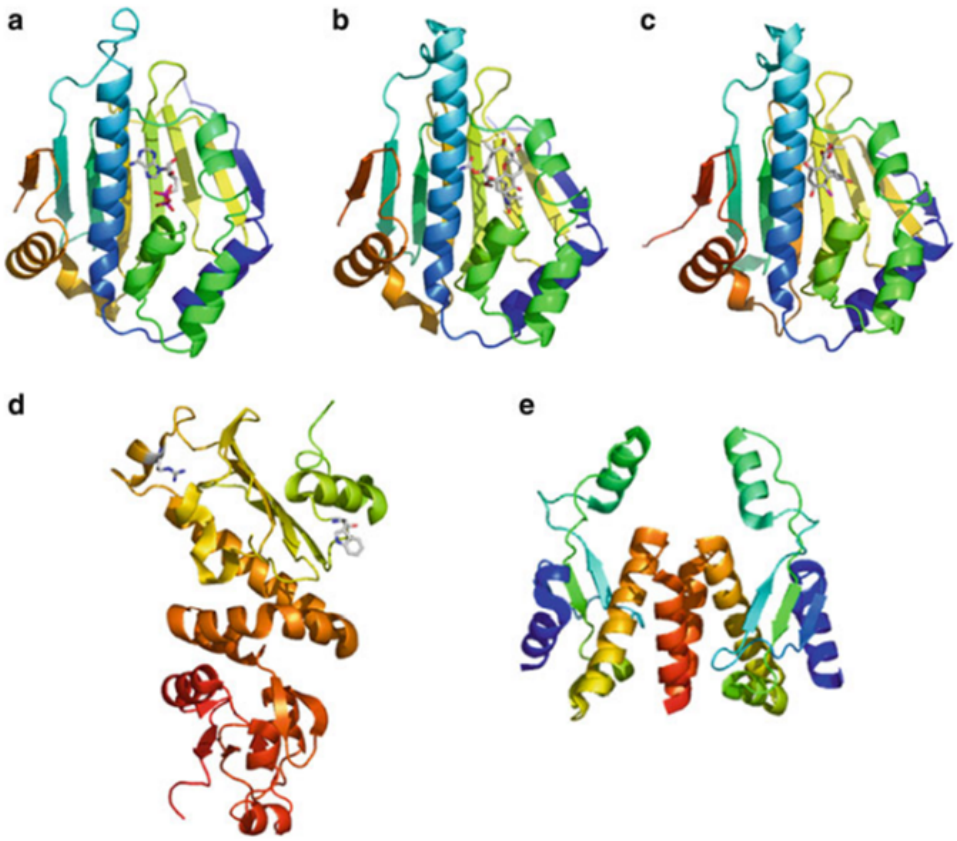


Fig:*(a) Structure of the N-terminal domain of human Hsp90 with ADP bound (pdb code: 1BYQ). The ribbon is coloured dark blue to red from N-terminus to C-terminus, and ADP is shown as a stick model.*

(b) Structure of the N-terminal domain of human Hsp90 with geldanamycin (GA) bound (pdb code: 1YET). The ribbon is coloured dark blue to red from N-terminus to C-terminus, GA is shown as a stick model.

(c) Structure of the N-terminal domain of yeast Hsp90 with Radicicol (RD) bound (pdb code: 1BGQ). The ribbon is coloured dark blue to red from N-terminus to C-terminus, RD is shown as a stick model.

(d) Crystal structure of the middle domain of yeast Hsp90 (pdb code:1HK7), the side chains of residues Phe300 and Arg380 are shown in stick models. The ribbon is coloured from N-terminus (yellow/green) to C-terminus (red).

(e) Crystal structure of the dimeric C-terminal domain of the E. coli HtpG (pdb code: 1SF8). The ribbons of the two chains are coloured the same from dark blue (N-terminus) to red (C-terminus). The dimerization interface forms a four-helical bundle (a-helices coloured in orange and red).

The first structure of a full-length construct of Hsp90 was published in 2006 by the Perl group. To crystallize the highly complex chaperone they engineered it such that it lacked the highly charged linker region between the N-terminal and the middle domains and contained a mutation to increase the ATPase activity by increasing the NTD dimerization. Thus capturing the Hsp90 in a closed state.

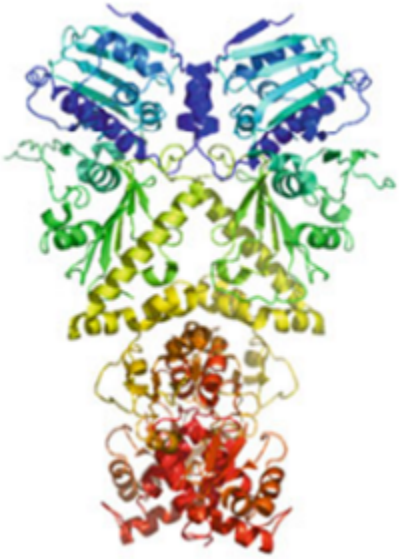


Fig: Structure published by the Pearl Group

ATPase Activity

Hsp90 ATPase Activity

In the beginning, there was a controversy over whether Hsp90 was an ATP-binding protein with ATPase activity or not, this was almost confirmed by the low affinity of Hsp90 towards ATP compared to the other ATPases. But later on, a certain variant of Hsp90 confirmed that Hsp90 did bind to and hydrolyse ATP and that it was essential for the function of Hsp90. To further prove this Hsp90-specific inhibitor geldanamycin was used to demonstrate that the

ATPase activity was detected from the Hsp90 and not due to some other contaminant, this also made geldanamycin an effective ATPase activity inhibitor.

Cochaperones

Now that it is well established that the ATPase activity of Hsp90 is highly regulated by cochaperone binding, post-translational modification and even client-protein binding. The effect of several cochaperones on the ATPase activity has been studied in detail and together with its structural work the mechanisms by which the cochaperones up-regulate or down-regulate the activity.

p23

p23 was the first complex with Hsp90 to be identified. It is known to bind to the N-terminal domains of Hsp90 and preferentially to the ATP-bound state, thereby stabilising the N-terminally dimerized form of the chaperone. But it has also been shown to bind to apo Hsp90 although that bond is weaker without the ATP. It inhibits the ATPase activity of the chaperone in ways unknown till now, although it most likely is simply by not allowing the release of ADP and phosphate.

PP5 (Protein Phosphate 5)

PP5 contains a TPR building site as well as a phosphatase domain that can bind to Hsp90 through its C-terminal. It is known to be involved in the regulation of tau protein. It gives important background on the cellular signalling pathways, the range of cellular processes, and the regulation of stress-induced signalling networks and cancer.

Cdc37

Cdc37 is an Hsp90 cochaperone required for the correct activation of many cellular kinases. It is a well-known cochaperone that has been shown to bind with the N-terminal of Hsp90. Cdc37 binds to the kinases either during or shortly after translation, thereby protecting them against proteasomal degradation. It is an essential protein that turns oncogenic when overexpressed thus making it an important therapeutic target.

Hop

Hop, Hsp70-Hsp90 organizing protein that comprises three TPR domains- TPR1, 2A and 2B. this cochaperone has been evaluated extensively, it is known to bind simultaneously with both Hsp70 and Hsp90 thereby acting as an adaptor protein, mediating the transfer of client proteins from Hsp70 machinery to Hsp90 system.

Aha1

Aha1 was first identified as a multi-copy suppressor of an inactive mutant of Hsp90 in yeast restoring wild-type-like growth rate it also stimulated the ATPase activity of Hsp90.

Hsp90 as Cancer Target

Many Hsp90 clients are known to be involved in multiple oncogenic pathways and Hsp90 is now a well-established target of anti-tumour and anti-proliferative drugs. Two natural products geldanamycin and benzoquinone have both been shown to have antiproliferative activity and target the ATP-binding site in the N-terminal domain of Hsp90, thereby inhibiting the essential ATPase activity and function of the chaperone. Inhibition of Hsp90 causes client proteins to undergo ubiquitination and subsequent degradation by the proteasome. Initial studies on geldanamycin derivatives validated Hsp90 as a cancer target and numerous studies have now been undertaken by both academic laboratories and pharmaceutical companies to develop further small molecule inhibitors of Hsp90 as therapeutic agents.

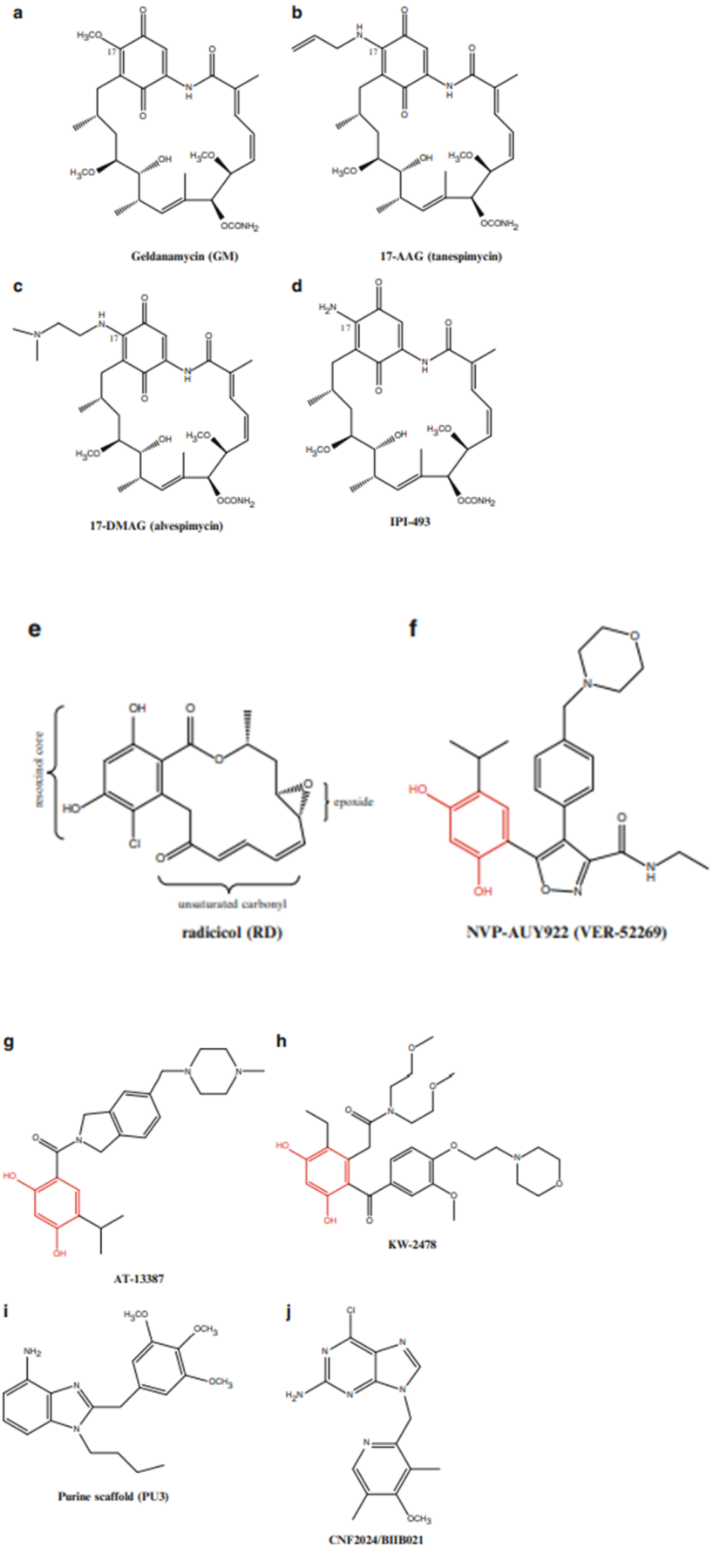
Geldanamycin (GA) was first identified in a screen aimed at identifying molecules capable of reversing the phenotype of v-src transformed cells. It has been shown to inhibit the ATPase activity Hsp90 competing with ATP for the ATP-binding site in the N-terminal domain. GA itself has poor solubility, limits in vivo stability and it is hepatotoxic, making it a poor drug candidate. However, substituents at the C17 position have improved properties. In particular, substitution with an allylamino group to produce 17-AAG resulted in an improved toxicity profile and 17-AAG has undergone extensive clinical trials. Although some of these prove to be of limited success recently better formulation and delivery

methods have proved encouraging. In particular, combination therapies of 17-AAG with other chemotherapeutics drugs and radiotherapy. Substitution at c17 has also produced another derivative, 17-DMAG which has improved the water solubility and oral bioavailability whilst retaining good anti-tumour activity, IPI-504 also known as retaspimycin has been shown to have improved pharmacokinetics and toxicity; in addition, IPI-493 a primary active has been trialled but shown to have poor pharmaceutical properties.

Macrocyclic lactone radicicol was identified very early as having anti-proliferative activity and targeting Hsp90, it has never been developed as a therapeutic agent as it was quickly established it was not stable in serum and had no in vivo activity because of the reactive epoxide and unsaturated carbonyl. However, it contains a resorcinol moiety which has been found in several other Hsp90 molecules that are in or entering, clinical trials. These were discovered by a variety of techniques including high throughput screening and fragment-based drug discovery not by modification of RD itself.

The first reported synthetic Hsp90 inhibitor was based on a purine scaffold and incorporated the features observed in the binding of ATP into Hsp90, most importantly a bent conformation. It has been extensively optimized in terms of its pharmaceutical properties, and a large number of such purine-based inhibitors are now in clinical trials. This includes CNF2124/BIIB021.

Peptidomimetics have also been developed which bend to the N-terminal domain of Hsp90, thereby inhibiting ATP binding and hydrolysis. Shepherdin is an example which was modelled based on the binding of the protein survivin to Hsp90, survivin being an anti-apoptotic and mitotic regulator. Shepherdin has been shown to destabilise Hsp90 inhibition more than normal calls and there is a preferential accumulation of the inhibitors in tumour cells. It has been proposed that this may be because Hsp90 in cancer cells is to a significant degree tied up in large multi-chaperone-client-protein complexes, and it has been suggested that these complexes have a higher affinity for the inhibitors than the Hsp90 in normal calls, in which a much smaller fraction is associated with cochaperones and clients.


a
b
c
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Geldanamycin (GM)
17-AAG (tanespimycin)
17-DMAG (alvespimycin)
IPI-493
resorcinol core
epoxide
unsaturated carbonyl
radicicol (RD)
NVP-AUY922 (VER-52269)
AT-13387
KW-2478
Purine scaffold (PU3)
CNF2024/BIIB021

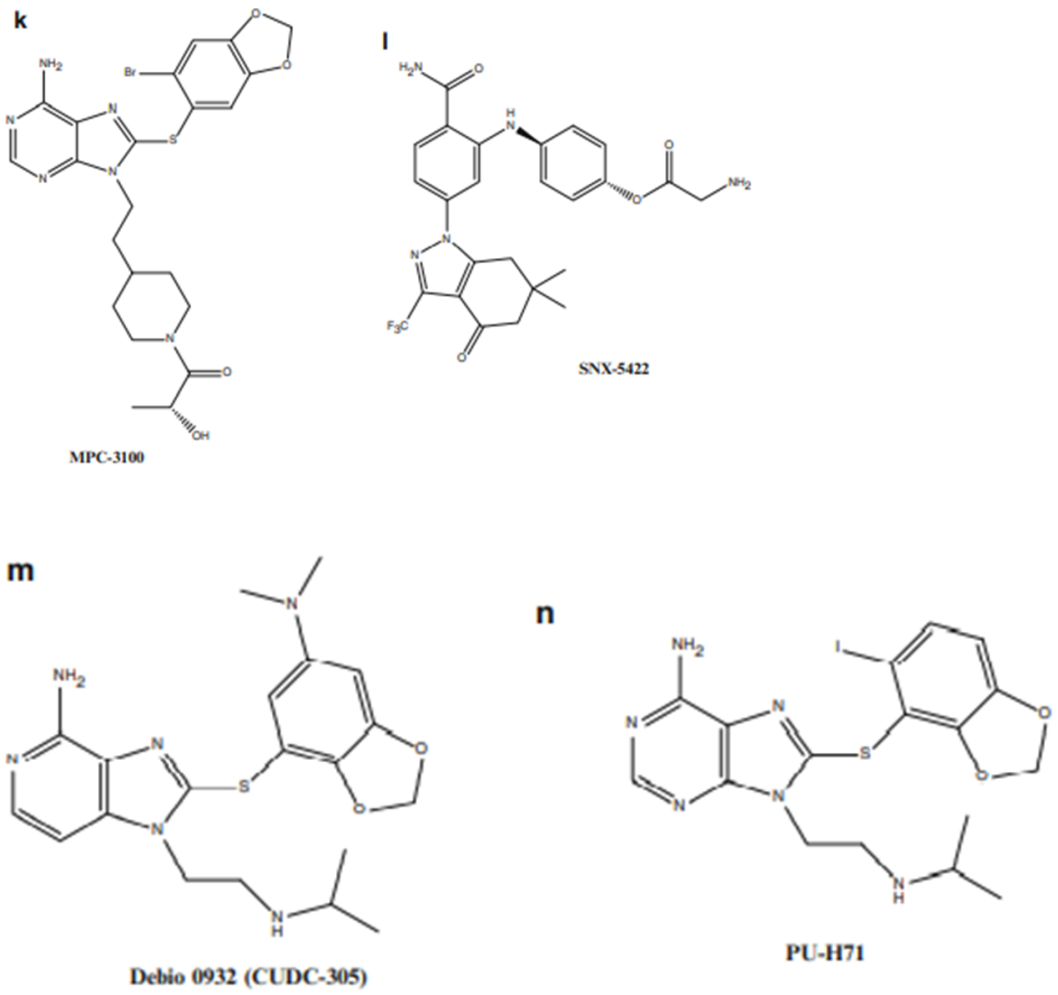
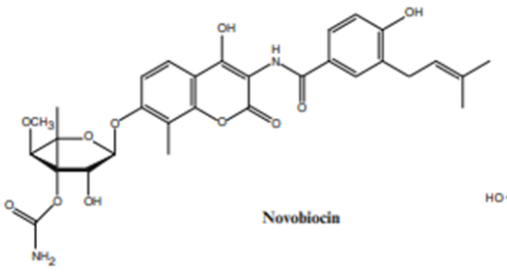


Fig: (a–d) Structures of geldanamycin (GM) and its derivatives including (a) GM (b) 17-AAG, (c) 17-DMAG and (d) IPI-493 which is a primary active, long-lived metabolite of 17-AAG. (e) Structure of the macrocyclic lactone radicicol (RD). (f) Structure of an isoxazole derivative NVP-AUY922/VER2296 containing a resorcinol moiety (highlighted in red) developed by Cancer Research UK. (g) Structure of AT-13387 developed using a fragment-based approach by Astex Therapeutics and containing a resorcinol moiety (highlighted in red). (h) Structure of KW-2478. (i) Structure of the first synthetic Hsp90 inhibitor PU3, based on a purine scaffold. (j) Structure of the purine-based inhibitor CNF 2024/BIIB021. (k) Structure of the purine-based inhibitor MPC-3100. (l) Structure of SNX-5422 a pyrazole-containing inhibitor. (m) Structure of Debio 0932. (n) Structure of PU-H71

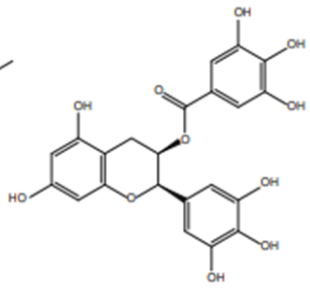
Several other small molecules have also been discovered that interact with and disrupt the activity of Hsp90 but do not bind to the ATP-binding site in the N-terminal domain. There is a growing interest in these as modulators of Hsp90 activity which can either be used in in

vivo studies of Hsp90 function or as potential therapeutics. Novobiocin is a coumarin antibiotic, it has long been known to bind to the C-terminal domain of Hsp90 although it is a weak bond. In addition to its potent activity against Gram-negative bacteria, it has also been shown to have antitumour activity against breast cancer cells. It does not affect the ATPase activity of Hsp90 but binds to a site in the C-terminal domain which results in the disruption of cochaperone binding and degradation of clients.

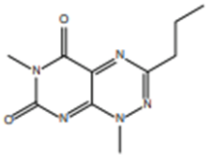
Epigallocatechin-3-gallate (EGCG), one of the polyphenolic components of green tea, has also been shown to bind to some of its clients C-terminal domain of Hsp90, disrupt its function and inhibit the activity of some of its clients including telomerase, some kinases and Ahr. In addition, there is also evidence that cisplatin can bind to the C-terminal domain of Hsp90 and it has been proposed that some of the anti-cancer activity of cisplatin may be due to Hsp90 inhibition.



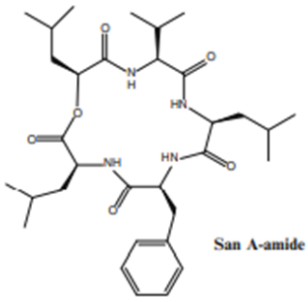
Novobiocin



Epigallocatechin-3-gallate (EGCG)



3-propylpyrimido[5,4-e][1,2,4]triazine-5,7-dione (C9)



San A-amide

A recently used novel approach to select and develop molecules which block the binding of TPR- domain cochaperones including Hop to Hsp90 and so inhibit the activity of Hsp90. Using this approach, compounds were identified that were active in vivo against a number of different cancer cell lines which resulted in a decrease in levels of Hsp90 clients such as

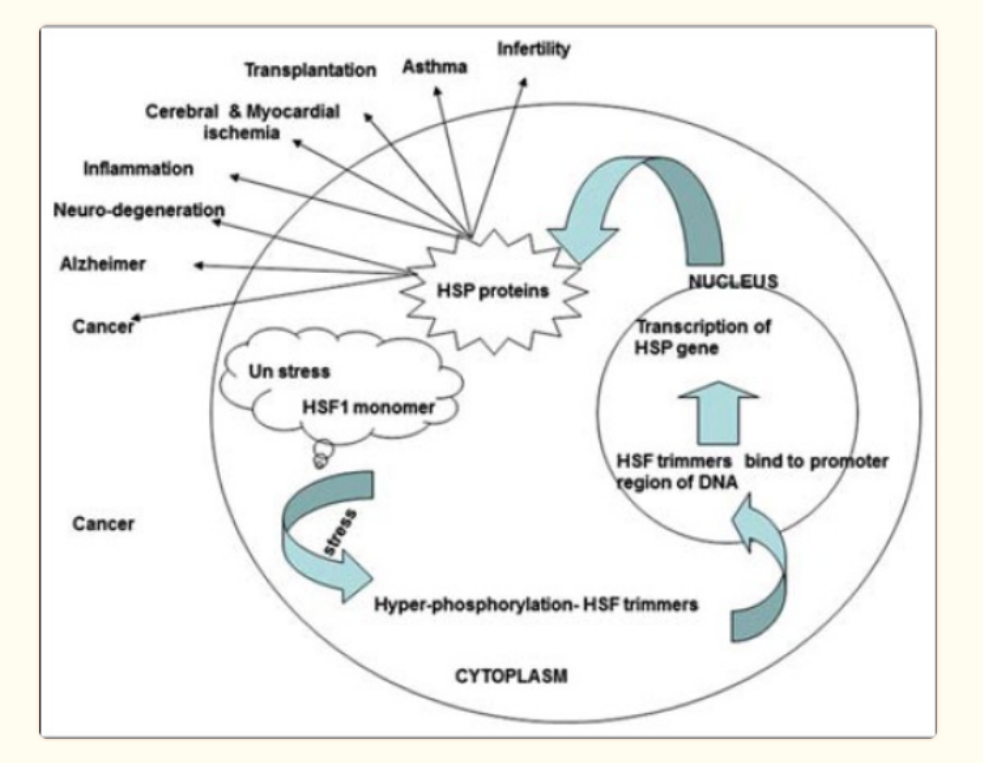
Her2 which is associated with breast cancer consistent with the action of the well-established inhibitors which bind to the ATP-binding site. In addition, they designed and engineered TPR proteins which bind to the C-terminal MEEVD motif of Hsp90 with higher affinities than Hop itself, thus acting as competitive inhibitors of Hop binding and reducing Hsp90 function in vivo. These proteins were also active against a breast cancer cell line and also reduced levels of Her2. One of the compounds from the AlphaScreen, HTS and C9 was recently shown to be effective in killing a number of different breast cancer cell lines. Of particular interest in this study was the fact that C9 does not up-regulate the expression of Hsp70, which occurs with the ATP-binding site inhibitors and which partially counteracts the beneficial effects of Hsp90 inhibition.

San-A-amide is another example of a small molecule inhibitor of Hsp90 action that specifically binds to a reason encompassing both the N-terminal and middle domain of Hsp90. It has been shown to disrupt allosterically the binding of proteins to the C-terminal domains of Hsp90, thus disrupting function and thereby affecting a subset of cancer-related pathways.

2.2 Hsp as therapeutic targets

Heat shock proteins are a type of molecular chaperons that are expressed during normal cell cycle and when the cell is under stressful conditions, thus extending the survivability of cells. Heat shock proteins (HSPs) are classified based on their molecular weight and function. One of such classes is the HSP90 which we will be talking exclusively about.
HSP90 has proven to be an ideal candidate for cancer diagnosis, prognosis and treatment. From various experiments already concluded, we can conclude that either inhibition or expression of HSPs gives a vast area of therapeutic targets against various diseases. This includes cancer.
When the cell is in an unstressed state, HSF(Heat shock factor) is present in the cytoplasm as a latent molecule. However, when the cell is under stress, HSF is hyperphosphorylated. It is then converted into phosphorylated trimers and binds DNA and translocated from cytoplasm to nucleus. However, they are downregulated once it reaches the nucleus due to

the HSPs and heat shock binding protein present in the nucleus. However, the induction of HSPs is transient and negatively impacts the protein homeostasis.



Of the HSPs, HSP90 is the most studied and abundant protein in eukaryotes. It is conserved in the organism throughout evolution and is essential for cell survival. HSP90 plays a very important role in protein folding and refolding. It is also responsible for stabilising various kinases that are responsible for malignant transformation of the cells.HSP90 can bind to several signalling proteins known as client proteins. These include cancer-relevant targets such as mutated p53, Bcr-Abl, Raf-1 and other kinases. Due to its ability to bind to its client proteins and to increase cell survivability, HSPs have also been found to promote resistance to anti-cancer therapies such as chemotherapy and radiotherapy.

CHAPTER 3

METHODOLOGY

3.1 Hsp90 Inhibitors

An **Hsp90 inhibitor** is a substance that inhibits the activity of the HSP90 Heat shock protein. Since Hsp90 stabilizes a variety of proteins required for the survival of cancer cells, these substances may have therapeutic benefits in the treatment of various types of malignancies. Furthermore, a number of Hsp90 inhibitors are currently undergoing clinical trials for a variety of cancers. Hsp90 inhibitors include the natural products geldanamycin and radicicol as well as semisynthetic derivatives 17-N-Allylamino-17-demethoxygeldanamycin (17AAG).

Heat shock protein 90 (Hsp90) family members are ATP-dependent molecular chaperones that regulate the stability and function of client proteins involved in the growth, survival, and adaptation of cancer cells to cellular stress. Hsp90 family proteins comprise four paralogs, each of which resides at different subcellular locations: Hsp90α/β in the cytoplasm, glucose-regulated protein 94 (Grp94) in the endoplasmic reticulum (ER), and tumor necrosis factor receptor-associated protein-1 (TRAP1) in mitochondria. Each Hsp90 paralog plays a crucial role in tumor progression, multidrug resistance, increased cell death threshold, and metastasis. Thus, simultaneous inactivation of all Hsp90 family proteins can compromise multiple tumorigenic pathways operating in different cellular compartments; this approach is very likely to increase anticancer activity above that afforded by paralog-specific inactivation of Hsp90 family proteins.

The Hsp90 family proteins Hsp90, Grp94, and TRAP1 are present in the cell cytoplasm, endoplasmic reticulum, and mitochondria, respectively; all play important roles in tumorigenesis by regulating protein homeostasis in response to stress. Thus, simultaneous inhibition of all Hsp90 paralogs is a reasonable strategy for cancer therapy. Analysis of The Cancer Genome Atlas database revealed that all Hsp90 paralogs were upregulated in prostate

cancer The purine scaffold derivative DN401 inhibited all Hsp90 paralogs simultaneously and showed stronger anticancer activity than other Hsp90 inhibitors.

Functions

Hsp90 inhibitors are divided into several groups according to the form of inhibition, which includes:

i) blocking the binding of ATP

ii) Decoupling co-chaperon/Hsp90

iii) Antagonism of client/Hsp90 association.

The benzoquinone ansamycins mimic ATP and bind to the nucleotide-binding pocket on the N-terminus of Hsp90, blocking the natural substrate ATP binding. Geldanamycin (GA) and its analogs specifically inhibit only Hsp90 (and its endoplasmic reticulum homolog grp94), but not other chaperones or other intracellular molecular targets. Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG) administration was shown to induce a pronounced downregulation of multiple Hsp90 protein clients and other downstream effectors, such as IGF-IR, Akt, IKK-α, IKK-β, FOXO1, Erk1/2, and c-Met, resulting in sequestration-mediated inactivation of NF-κB, reduced cell proliferation and decline of cell motility. The inhibition of Hsp90 with 17-AAG and another ansamycin derivative 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) downregulated B-Raf, decreased cell proliferation, reduced Mek/Erk signaling activation and induced a significant inhibition of telomerase activity in melanoma cells. The Hsp90 inhibitor 17-AAG enhanced significantly the radiation sensitivity and induced apoptosis in the oral squamous cell carcinoma cell line SAS/neo which has a wild-type p53. On the other hand, the radiation-sensitizing effect of 17-AAG was limited in the SAS/Trp248 cells which have a mutated p53. 17-AAG was also shown to inhibit ATP-binding cassette sub-family G member 2 (ABCG2) upregulation, thereby reversing the ABCG2-mediated multidrug resistance (MDR) and in this regard can be used as a chemosensitizer for the treatment of esophageal cancer.

Results:

Unsatisfied with the results of N-terminal inhibitors in experimental and clinical trials arising from the increased toxicity and resistance induced by heat shock response led to the development and characterization of novel C-terminal inhibitors of Hsp90. Novobiantibioticbiotic of coumarin group) and its analogues (clorobiocin and coumermycin A1) have been generated as agents for the treatment of bacterial infections with multi-resistant gram-positive bacteria such as *Staphylococcus aureus* and *S. epidermis*. Coumarin antibiotics were reported to bind strongly to gyrase B (DNA topoisomerase II) but also weakly to the Hsp90 C-terminal nucleotide-binding site followed by induced degradation of Hsp90 client proteins such as v-Src, Raf-1, ErbB2, and mutant p53. Moreover, novobiocin disrupted the interaction of both the cochaperons p23 and Hsp70 with the Hsp90 complex. Structural modification of this compound has led to analogues with 1,000-fold greater efficacy in antiproliferative assays against various cancer cell lines.

3.2 Hsp based immunotherapy

Several studies have demonstrated that hsps/grps can act as vaccines when purified from diseased tissues (e.g. cancers). It has been demonstrated that hsps purified from tumours can elicit antigen-specific cytotoxic T cell (CTL) responses to the associated peptides chaperoned in the cancer cell by the hsp. The ability of hsps/grps to interact with APCs is expected to contribute to their effectiveness as vaccines.

Immunogenicity of HSPs

1. Tumour-derived hsp vaccines

Tumour-derived heat shock protein (HSP) vaccines are a type of cancer immunotherapy that utilizes heat shock proteins, which are proteins that are overexpressed in cancer cells, as a source of tumour antigens to stimulate an immune response against cancer.

HSPs as a source of tumour antigens is that they may represent a more complete and personalized set of tumour antigens than other types of cancer vaccines, as they are derived

directly from the patient's own tumour cells. Additionally, HSP vaccines are thought to be particularly effective at stimulating T-cell responses, which are critical for the immune system's ability to target and destroy cancer cells.

2. Hsp-protein-based vaccines

This new approach uses recombinant hsps non-covalently bound to a well-known, full-length, tumour antigen in vitro as a vaccine. This method uses the hsp's natural chaperoning property to form a natural hsp-protein antigen complex in vitro, usually by using heat shock as the coupling agent. This method yields a highly concentrated vaccine preparation that targets a specific protein antigen. More importantly, the adjuvant activity of mammalian hsps would aid in eliciting strong immune responses against the HSP-associated antigen in the absence of any additional adjuvant. Because whole proteins typically contain a large reservoir of potential antigenic epitopes that CD4/CD8 T cells can access. Lastly, this approach simplifies the vaccine production process since surgical specimens are not required. Preparation of such vaccines would be much less labour intensive than that of tumour-derived hsp and would be available in unlimited quantity. Currently, the vaccine efficiency of the hsp110 complex has been investigated in the laboratory.

3. Hsp-based DNA vaccines

This approach uses hsps in the form of chimeric DNA through the linkage of known antigen genes to hsp, which potentially increases the potency of DNA vaccines by a yet-to-be-identified mechanism. One significant advantage of DNA vaccines is the ability of vaccines to endogenously generate a CTL response against the antigen is important because it is difficult to induce a CTL response. CTL response induced by protein-based vaccines (excluding hsp vaccines, which can induce strong CTL responses). Another benefit of DNA vaccines is their durability and ease of preparation. Vaccines containing human papillomavirus type 16 E7 and hsp70 fusion genes have been shown to have preventive and therapeutic effects against an established tumour via CD8-dependent pathways.

CHAPTER 4
CONCLUSION

In conclusion, heat shock proteins (HSPs) play a critical role in maintaining protein homeostasis in normal cells but are often upregulated in cancer cells where they contribute to tumour growth, survival, and resistance to therapy. Targeting HSPs for cancer treatment has emerged as a promising therapeutic approach, as inhibiting the function of HSPs can lead to the degradation of oncoproteins and cause cancer cell death. Additionally, HSPs have been shown to be involved in the presentation of cancer antigens, making them attractive targets for cancer immunotherapy.

The approaches to the use of hsps in cancer immunotherapy, described in the chapter, are still in various stages of development. Only one has is being actively examined in clinical trials, i.e. tumour-derived hsp preparations. The examination of some of the other approaches is anticipated, if not already underway at the time of writing. Hsp-based immunotherapy (individual tumour-specific and tumour-associated antigen-specific) may be applicable in a broad range of patients and may also provide benefits as an adjuvant to current standard cancer therapies. Like other approaches in cancer immunotherapy, hsp vaccines, are not effective in all patients and might only increase the survival time and improve the quality of life. As is the case with cancer immunotherapy in general, hsp-based vaccines may require improvement to generate more potent therapeutic cancer vaccines. Since the immune system consists of several different and interacting elements, a combinational therapy utilizing other approaches to immunomodulation together with hsp-based vaccination.

However, targeting HSPs for cancer treatment also poses challenges, including potential impacts on normal cellular function and the potential for cancer cells to develop resistance to HSP inhibitors over time. Nonetheless, the growing body of research on HSPs in cancer treatment has provided valuable insights into the mechanisms underlying cancer development and the potential for developing new and effective cancer therapies. Further research is needed to fully understand the role of HSPs in cancer and to identify safe and effective strategies for targeting these molecules in cancer therapy.

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IMMUNOTHERAPY OF CANCER USING HEAT SHOCK PROTEINS

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Heat Shock Protein 90 (Hsp90) Expression and Breast Cancer

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