Summary of Doctoral Thesis

Design and Development of Nano-formulation for Chaperone (Hsp90) Targeted Anticancer Drugs Using Biopolymers

Rochani Ankit Kanaiyalal
Student ID: 4R10140003

Doctoral Course
Course of Bio Nano Fusion Science
Graduate School of Interdisciplinary New Science
Toyo University, Japan
Academic Year 2016

Summary

Heat shock protein 90 (Hsp90) is chaperone that helps in protein folding process. . Structurally, each monomer of the dimeric Hsp90 consists of a) N-terminal domain, b) Charged linker region and c) C-terminal domain. In cancer, Hsp90 serves as a hub for repair of the damaged client proteins that are associated to cancer growth and development. Inhibition of Hsp90 shuts down the molecular machinery that is essential for survival and development cancer cells. As a result Hsp90 is considered as a potential anticancer drug target for variety of cancers. Most common focus of Hsp90 targeted therapy is to develop inhibitor that binds to ATP binding site of N-terminal domain. The protein-folding pathway of Hsp90 works mainly due to binding of ATP to N-terminal site of protein. Competitive association of ligands to ATP binding site of Hsp90 causes cancer cell death due to disruption in the function of Hsp90. 17-allyl amino 17-demethoxy geldanamycin (17AAG) is an N-terminal binding Hsp90 inhibitor. The discovery 17AAG in 1995 lead to the foundation of Hsp90 targeted cancer therapy. The molecule entered advanced clinical trials to fight against variety of cancers. Since the advent of the era of Hsp90 inhibitors there nearly fifteen Hsp90 targeting molecules that have entered clinical trials against variety of cancer conditions. Off these inhibitors, 17AAG and Luminespib have been extensively studied for their pharmacological properties, safety and efficacy. Further, it is also been reported that Hsp90 inhibitors may work effectively as combination therapy for certain cancer conditions. There are recent studies that suggest that Hsp90 inhibitors may also work extremely well with magnetic hyperthermia induced by Fe_3O_4 magnetic nanoparticles (MNPs).

Most Hsp90 inhibitors encounter the problem of patient non-compliance due to their water insolubility and variable effects in various cancers. In order to solve this

problem we developed bio-polymeric nanoformulation to explore the use of Hsp90 inhibitors **a)** in combination with magnetic hyperthermia and **b)** bovine serum albumin loaded second generation Hsp90 inhibitor (Luminespib) that can be used against various cancers. We have subdivided present work into five main chapters and conclusion chapter. Following is the brief summary for each chapter:

Chapter I: Heat Shock Protein 90 Targeted Nano Anti-Cancer Therapy

Cancer disease has an extremely complex dynamics. Nearly two decades of Hsp90 targeted drug discovery has shown that the mono-therapy with Hsp90 inhibitors seems to be relatively ineffective compared to combination treatment due to several cellular dodging mechanisms. In this article we have tried to analyze and review the Hsp90 and m-TOR mediated drug resistance mechanisms. By using this information we have discussed about the rationale behind use of drug combinations that includes both or any one of these inhibitors for cancer therapy.

Currently, biodegradable nano vector (NV) loaded novel drug delivery systems (NDDS) have shown to resolve the problems of poor bioavailability. NVs of drugs like Paclitaxel, Doxorubicin, Daunorubicin and others have been successfully introduced for medicinal use. Drug delivery systems are considered as largest market accounting for 80% sales in nanomedicine sector. It was assumed that nearly 30% of value a drug is added by NDDS. In 2006, it was reported that nanomedicines were estimated to contribute nearly 20 billion USD in the health care sector by 2012 in EU. However, the global nanomedicine market in 2009 was estimated to be 53 billion USD. On the other hand European technology platform (ETP) predicted that overall nanomedicine revenue would be in the range of 97 to 126 billion USD by 2016. It is estimated that the anticancer nanomedicine market is estimated to cover 33% of the

total nanomedicine market in 2014. Hence, looking at the success of NVs, in this article we have also discussed about the progress made in the delivery of biodegradable NV loaded with Hsp90 and m-TOR targeted inhibitors in combinations regime. We have also discussed the possible ways by which the market success of biodegradable NVs can positively impact the clinical trials of anti-Hsp90 and m-TOR combination strategy.

Chapter II: Instrumentation and Experimental Setup

In our work we have synthesized nanoformulations of Hsp90 inhibitors for anticancer efficacy. In the present chapter, we have summarized basic principle for the instruments like scanning electron microscopes (SEM), transmission electron microscope (TEM), X-ray photoelectron spectroscopy (XPS), U.V visible spectroscopy, magnetic hyperthermia coil system and others.

Chapter III: Dual mode of cancer cell destruction for pancreatic cancer therapy using Hsp90 inhibitor loaded polymeric nano magnetic formulation

17- N-allylamino- 17-demethoxygeldanamycin (17AAG) has shown to provide considerable anticancer efficacy against pancreatic cancer when used in combination with other drugs. Further, magnetic hyperthermia has also shown to have promising effects against pancreatic cancer in combination with known cyto-toxic drugs under both target and non-targeted scenarios. Hence, in order to enhance the efficacy of 17AAG against pancreatic cancer, we developed poly (lactic-co-glycolic acid) (PLGA) coated, 17AAG and Fe_3O_4 loaded magnetic nanoparticle formulations by varying the relative concentration of polymer. Our study shows that 1:1:10 drug loaded polymeric MNPs (DMNP) formulation had relatively better results for particle

characterization studies. It was observed from the drug release profile that the amount of drug released from 1:1:10 formulation was relatively more compared to 1:1:20. We also found that polymer concentration affects the magnetic strength formulations. We evaluated drug and magnetic hyperthermia induced in vitro cytoxicity for our nanoformulations. We were also able to see that our aqueous dispensable formulations were able to provide anti-pancreatic cancer activity for MIA PaCa-2 cell line in dose and time dependent manner in comparison to mice fibroblast cell lines (L929). Moreover, the in-vitro magnetic hyperthermia against MIA PaCa-2 provided proof principle that our 2-in-1 particles may work against cancer cell lines effectively. This study is a first step towards the improvement over the currently available regime for pancreatic cancer therapy. Hence, we believe that our study can be considered for the further molecular and in vivo evaluations.

Chapter IV: Molecular Modeling for Interaction of Anticancer drugs with Protein Based Carriers.

In the present study we have used Lamarkian genetic algorithm based AutoDock for the identification of probable binding site for Luminespib on BSA. However, since there is no reference ligand to map the binding sites on BSA, we scanned entire surface of the protein using blind docking method to find an unbiased binding site for Luminespib. We believe that further analysis of inhibition constants (Ki), % frequency and structure of the conformations obtained with grid size of a) 60 x 60 x 60, b) 90 x 60 x 80 with PM6 partial charge calculations should provide a more reliable information about the binding site for Luminespib on BSA. Perhaps, the wet lab experiments like fluorescent quenching and XPS may also confirm the binding of Luminespib to BSA.

Chapter V: Luminespib Loaded Protein Based Nanoformulation for Hsp90 Targeted Cancer therapy

Bovine serum albumin (BSA) is an important and abundant protein for buffering various biological functions. BSA has also been extremely useful biomaterial for preparation of NPs that can be used as novel drug carrier for anticancer drugs. BSA based NPs have be extremely successful and are translated for clinical use as anticancer drugs. For example Paclitaxel loaded BSA NPs called as Abraxane by Abraxis pharmaceuticals have entered for clinical use against pancreatic cancer, breast cancer, lung cancer and others. Hence, use of the BSA based platform for formulation of Hsp90 inhibitors may also prove useful. Luminespib (NVP-AUY922) is a new generation Hsp90 inhibitor that was introduced recently for advance clinical trials as mentioned previously. However, like many other Hsp90 inhibitors, it is also a hydrophobic molecule. We have developed BSA NP formulation for Luminespib using classical desolvation method. We found that Luminespib interacts by non-covalent reversible interactions with BSA to form drug loaded BSA conjugated NPs (DNPs). Our study suggests that Luminespib binds to domain I of BSA by forming thirteen reversible hydrophilic and hydrophobic interactions. We have also confirmed that binding of Luminespib to BSA using fluorescence quenching experiment. We found that with the increase in relative concentration of Luminespib there was reduction in fluorescence intensity. This clearly indicates binding of drug to BSA protein. We further confirmed binding of Luminespib to BSA by using XPS. We found presence of signature peaks associated to drug in Luminespib-BSA NPs in comparison to native or blank BSA NPs. Our U.V spectroscopy study suggests that encapsulation efficiency and drug loading in Luminespib-BSA nanoconjugates was

found to be 48.22 ±1.948 % and 4.28±1.94 % respectively. We believe that this formulation can be used for further evaluation of the anticancer therapy.

Chapter VI: Conclusion

Our analysis suggests that NP based NDDS can help in developing water dispensable colloidal dispersion for hydrophobic Hsp90 inhibitors. It was observed that Hsp90 inhibitors works better in certain cancer as single therapy while in others they work better in combination regime. Hence, cancer treatment seems to be done on a personalized basis. It was observed that 17AAG proved to be effective in combination therapy. As a result our drug loaded polymeric magnetic nanoformulation showed Hsp90 inhibitor and magnetic hyperthermia induced cytotoxicity in a concentration dependent manner. We altered relative polymeric concentration to give two types of formulations as 1:1:20 and 1:1:10. We observed that nanoformulation with ratio of drug to Fe_3O_4 to polymer as 1:1:10 exhibited relatively better pharmacological response against pancreatic cancer. Further, we have also developed Luminespib loaded bovine serum albumin NPs. Here, we used drug to polymer ratio as 1:10. We found that the drug forms non-covalent interactions with BSA to form drug-BSA nanoconjugates. We also observed sustained release of drug from our nanoformulation. The nanoparticles were found to be of size around 222 nm. Our studies clearly indicate that the smart nanoformulation of hydrophobic Hsp90 inhibitors can help increasing the patient compliance by lowering the dose related toxicity that is mostly attributed to the classical DMSO based formulations.