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学 位 の 種 類	博士（バイオ・ナノサイエンス融合）		
報告・学位記番号	甲第420号（甲バ第８号）		
学位記授与の日付	平成29年３月25日		
学位記授与の要件	本学学位規程第３条第１項該当		
学 位 論 文 題 目	Design and Development of Nano-formulation for Chaperone (Hsp90) Targeted Anticancer Drugs Using Biopolymers (和訳：バイオポリマーを用いたシャペロン（Hsp90）標的型抗癌剤ナノ粒子の設計と開発)		
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【論文審査】Review of the thesis

Heat shock protein 90 (Hsp90) is a dimeric molecular chaperone that helps in protein folding process. In cancer, Hsp90 serves as a hub for repair of the damaged client proteins that are associated to angiogenesis, signal transduction pathways. Inhibition of Hsp90 shuts down the molecular machinery that is essential for survival and development cancer cells. Most common focus of the developing Hsp90 targeted therapy is to develop inhibitor that can disrupt the ATPase activity of Hsp90 and damage the essential client protein by activation of proteasome degradation. Hsp90 inhibitors bind to the N-terminal subdomain of the protein. The protein-folding pathway of Hsp90 works mainly due to association of ATP to the 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. The discovery of 17-allyl amino17-demethoxy geldanamycin (17AAG) lead to the foundation of the developing Hsp90 targeted cancer therapy. The molecule entered advanced clinical trials to fight variety of cancer. Most Hsp90 inhibitors encounter the problem of patient non-compliance due to their water insolubility and variable effects in various cancer types. In order to solve this problem; Ankit developed bio-polymeric nanoformulation to explore the use of Hsp90 inhibitors **a**) in combination with magnetic hyperthermia and **b**) bovine serum albumin (BSA) loaded second generation Hsp90 inhibitor (Luminespib) against

variety of cancer conditions like breast cancer along with pancreatic cancer. Based on the work, Ankit wrote the Thesis **"Design and Development of Nano-formulation for Chaperone (Hsp90) Targeted Anticancer Drugs Using Biopolymers"**. Ankit has subdivided present work into six main chapters including the conclusion chapter.

Chapter I is based on the review work about the Heat Shock Protein 90 Targeted Nano Anti-Cancer Therapy. He did a very good review work and comprised the chapter by providing all relevant information about previous works based Hsp90 in anti-cancer field. Suboptimal chemotherapy of anticancer drugs may be attributed to a variety of cellular mechanisms, which synergize to dodge the drug responses. 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 chapter; Ankit tried to analyze and review the Hsp90 and m-TOR mediated drug resistance mechanisms. By using this information Ankit developed the rationale behind the use of drug combinations that includes both or any one of these inhibitors for personalized 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. Hence, looking at the success of NVs, Ankit also discussed about the progress made in the delivery of biodegradable NV loaded Hsp90 and m-TOR targeted inhibitors in multiple drug combinations. Ankit 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.

In Chapter II; Ankit described about the Instrumentation and Experimental Setup he used in his experiments. In order to characterize the synthesized nanoformulations he used scanning electron microscopes (SEM), transmission electron microscope (TEM), X-ray photoelectron spectroscopy (XPS), U.V visible spectroscopy, magnetic hyperthermia coil system and others. He also summarized basic working principles of the instruments that were used for performing these experiments.

In Chapter III; Ankit mentioned about the dual mode of cancer cell destruction for pancreatic cancer therapy using Hsp90 inhibitor loaded polymeric nano magnetic formulation. Heat Shock Protein 90 (Hsp90) has been extensively explored as a potential

drug target for cancer therapies. 17- N-allylamino- 17-demethoxygeldanamycin (17AAG) was the first Hsp90 inhibitor to enter clinical trials for cancer therapy. However, native drug is being shown to have considerable anticancer efficacy against pancreatic cancer when used in combination therapy regime. Further, magnetic hyperthermia has 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, Ankit developed poly (lactic-co-glycolic acid) (PLGA) coated, 17AAG and Fe_3O_4 loaded magnetic nanoparticle formulations by varying the relative concentration of polymer. He found that polymer concentration affects the magnetic strength and physicochemical properties of formulation and 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.

In Chapter IV Ankit described about the Molecular Modelling for Interaction of Anticancer drugs with Protein Based Carriers. Docking simulations have played important role in the theoretical understanding of the binding of ligand with protein of interest. In the present study he has used Lamarkian genetic algorithm for the identification probable binding site of Luminespib on BSA. However, since there is no reference ligand to map the binding sites on BSA, he scanned entire surface of BSA to find an unbiased binding site for Luminespib. He suggests 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 conformation or binding site for Luminespib on BSA.

In Chapter V Ankit describes about the Luminespib Loaded Protein Based Nanoformulation for Hsp90 Targeted Cancer Cell Destruction for Pancreatic and Breast Cancer Therapy. Drugs targeting Heat shock protein 90 (Hsp90) has been extensively explored for the anticancer potential in advanced clinical trials. However, water insolubility of Hsp90 inhibitors is one of the factors that have limited their development. It has been noticed that bovine serum albumin (BSA) nanoparticles (NPs) serves as carrier for anticancer drugs, which have been extensively explored for their therapeutic efficacy against cancers. Luminespib (NVP-AUY922) is a new generation Hsp90 inhibitor

that was introduced recently. It is one of the most studied Hsp90 inhibitor for variety of cancers in Phase I and II clinical trials; similar to its predecessors like ansamycin class of molecules. However, like many other Hsp90 inhibitors in advanced clinical trials it is also hydrophobic in nature. Hence he developed BSA NP carrier for the aqueous dispensability of Luminespib. He found that Luminespib interacts by non-covalent reversible interactions with BSA to form drug loaded BSA conjugated NPs (DNPs) and this formulation can be used for the cancer therapy.

Ankit concludes the Chapter VI with Conclusions of his work based on his nanoformulations. Nanoformulation of known anticancer drugs in market like Doxil, Abraxane and others have set a benchmark for the success of NPs as NDDS. Nanoformulation market has been increased substantially in last few decades and it is projected to keep increasing in upcoming years. 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, Hsp90 inhibitor loaded polymeric magnetic nanoformulation exhibited Hsp90 inhibitor and magnetic hyperthermia induced cytotoxicity in a concentration dependent manner. Ankit altered relative polymeric concentration to give two types of formulations as 1:1:20 and 1:1:10. Nanoformulation with ratio of drug to Fe3O4 to polymer as 1:1:10 exhibited relatively better pharmacological response against pancreatic cancer. He developed NVP-AUY922 loaded bovine serum albumin NPs. In this work drug to polymer ratio kept as 1:10. He found that the drug forms non-covalent interactions with BSA to form drug-BSA nanoconjugates. It showed a time dependent release of drug from our nanoformulation. The nanoparticles were found to be of size around 222 nm. His 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.

【審査結果】Summary and decision

The thesis entitled **"Design and Development of Nano-formulation for Chaperone (Hsp90) Targeted Anticancer Drugs Using Biopolymers"** focused on the synthesis of bio-polymeric (PLGA) nanoformulation to explore the use of Hsp90 inhibitors **a)** in combination with

magnetic hyperthermia and **b)** bovine serum albumin (BSA) loaded second generation Hsp90 inhibitor (Luminespib) against variety of cancer conditions like breast cancer along with pancreatic cancer. The results shown in the thesis are outstanding from an international point of view and the significant points in the present study are summarised below;

(1) Heat shock protein 90 (Hsp90) serves as hub for repair of the damaged proteins that are associated to angiogenesis, signal transduction pathways. It helps the cancer cells to proliferate hence cancer research world is trying to develop a method to inhibit Hsp90 that will shut down the molecular machinery which is essential for survival of cancer cells.

(2) Discovery of 17-allyl amino17-demethoxy geldanamycin (17AAG) which is an inhibitor of Hsp90 lead to the foundation of the developing Hsp90 targeted cancer therapy.

(3) Since 17AAG is hydrophobic its very difficult to deliver against cancer cells effectively. Hence Ankit developed a biopolymeric formulation of 17AAG for making it highly hydrophilic.

(4) By using biopolymeric combination of 17AAG and magnetic nanoparticles he could kill pancreatic and breast cancer cells.

(5) He also developed a BSA coated Luminespib (NVP-AUY922) which is a new generation of Hsp90 inhibitor along with magnetic nanoparticles. This new drug is highly effective against breast and pancreatic cancer cells.

(6) By using docking simulations, Ankit proposed the binding sites of Luminespib with BSA. This confirms that even when during the interaction of Luminespib with BSA, it keeps its chemical properties and is very effective in killing cancer cells.

(7) The present results of nanodrugs formulations are really very good in killing cancer cells effectively.

The results obtained by the present doctoral study have been highly appreciated by pharmaceutical science societies. Ankit published two first-authoring papers in international journals (Journal of Pharm. Sci. and Int. J. Pharm). He also presented many papers in International seminars too.

Judging by the results shown in the thesis and the number of international papers published so far, the level of the present research results is definitely high by international standards and the present results based on nanodrug formulations may

provide great contributions to the development of new methodologies and strategies to kill cancer effectively; particularly pancreatic cancer; which is currently having lowest survival rate. In conclusion, the thesis is considered to be a high quality, high standard one by international standards.