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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419号（甲バ第７号）		
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学　位　論　文　題　目	Therapeutic polymeric nanoparticles for targeting and destruction of cancer stem cells（CSCs） （和訳：がん幹細胞のみを標的とし破壊する治療用ポリマーナノ粒子の開発）		
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【論文審査】Review of the thesis

Nanotechnology has been able to extend a wide range of therapeutic advancements in the frontiers of cancer therapy research. Even though breakthrough progress has been made in the domain of current cancer research; the menace of the disease still persists. Accumulating evidences have suggested the presence of a subpopulation of cells that exists within tumors called as the cancer stem cells (CSCs) that exhibit resistance to conventional chemotherapeutics and unique growth resilience owing to the possession of distinctive properties.

Ankita Borah's thesis entitled **"Therapeutic polymeric nanoparticles for targeting and destruction of cancer stem cells (CSCs)"** underlies the approach of development of a prospective strategy to target CSCs with the aid of nanotechnology. Since CSCs possess certain properties specific to them, she was interested to exploit three aspects in order to target these fractions of cells. ① Hedgehog pathway GLI Antagonist (GANT61), known to have superior anti-cancer effects over existing Hedgehog pathway inhibitors and encapsulated inside a biocompatible polymeric Nano carrier. ② Bio-functionalized the GANT61 nanoformulation with an aptamer to achieve active targeting in cancer cells. The targeted aptamer GANT61 nanoformulation imparted cytotoxicity towards cancer cells

even at a low dose in comparison to the non-targeted GANT61 nanoformulation. ③ To achieve complete elimination of tumor mass by killing the bulk tumor cells as well as the cancer stem cells, she further incorporated a dual drug combination therapy by encapsulating GANT61 and curcumin inside PLGA nanoparticles, which concludes the final step of the research work.

Akita has divided the thesis into six chapters that is described as below:

Chapter 1 is the review work entitled "Therapeutic Strategies and Application of Nanotechnology for Elimination of Cancer Stem Cells: Introduction & Review of Literature" which discusses regarding the various aspects of CSCs that are being targeted currently to develop anti-CSC therapies and application of nanotechnology for the improvement of such therapies. The chapter is further sub-divided into two sections. Section 1.1 reviews the potential therapeutic targets that comprises of (i) targeting surface markers, (ii) targeting tumor microenvironment, (iii) targeting ABC transporters, (iv) targeting self-renewal pathways with experimental findings to target and eliminate CSCs. Section 1.2 discusses the advantages of application of nanotechnology for overcoming the hurdles often associated with the existing CSC therapies. Current nanomedicine approaches that are being developed to target CSCs include (i) development of drug delivery carriers, (ii) targeting drug resistant genes and (iii) targeting CSCs niche.

Chapter 2 comprise of the principle and operation of "Instrumentation" techniques that Ankita has utilized in her work. She described about various techniques such as SEM, TEM, XPS, FTIR and others to characterize the nanoparticles. Since the application of the therapeutic nanoparticles is in cancer therapy, she carried out in vitro cell culture studies in cancer cell lines that comprises of cytotoxicity assay, cellular uptake, optical microscopy, and others to demonstrate that the nanoparticles were successful in imparting cytotoxic effects to cancer cells.

Chapter 3 is entitled "Poly-lactic-co-glycolic acid Nanoformulation of Small Molecule Antagonist GANT61 for Cancer Annihilation by Modulating Hedgehog Pathway". In this chapter, she had employed the first strategy to target one of the self-renewal pathways aberrantly active in CSCs, which is the Hedgehog signalling pathway. She utilized a

recently discovered small molecule antagonist called as the Gli Antagonist (GANT61) that blocks the GLI1 protein in the downstream scenario of the pathway. GANT61 is limited for use in clinical purposes due to its poor solubility and bioavailability. This could be overcome by encapsulating the drug inside a polymeric nanoparticle. She had used FDA approved PLGA for the formulation of GANT61 due to its biocompatibility and biodegradability properties. She synthesized GANT61 PLGA NPs that had improved aqueous solubility compared to the free drug and had an average size of 250 nm. Due to its encapsulation inside PLGA NPs it conferred sustained release of the drug, which will have prolonged toxic effects in the cancer cells.

Chapter 4 describes about "CD133 Aptamer Targeted Delivery of GANT61 PLGA Nanoparticles to Colorectal Cancer Cells". The earlier synthesized GANT61 PLGA NPs were further modified to mediate aptamer based targeting of CSCs surface biomarkers for better killing of cancer cells. Since aptamers are small oligonucleotides having high target specificity, conjugation of these moieties onto the surface of GANT61 PLGA NPs would be a pragmatic approach to target the CSCs surface biomarkers. Ankita conjugated A15 aptamers onto the surface of GANT61 PLGA NPs that specifically targets AC133 epitope of the CD133 protein expressed by the CSCs in various solid tumors as reported. The conjugation process was carried out by EDC/NHS technique and the functionalized nanoparticles (A15-GANT61-PLGA NPs) were analyzed for its anti-cancer activity in colon cancer cells, which are reported to have a high expression of CD133.

Chapter 5 titled "PLGA Nanoparticles that Co-delivers GANT61 and Curcumin for Enhanced Anti-Tumor Activity in Breast Cancer" discusses the final and third strategy that Ankita has employed to develop dual drug combination therapy encapsulating GANT61 and curcumin in PLGA NPs. This novel nanoformulation would be an attractive approach to target the breast cancer tumor mass, which comprises of cancer cells and CSCs in a single lethal shot. The incorporation of curcumin provides an added advantage to monitor the cellular uptake of the NPs due to its inherent fluorescent nature and also possess anti-cancer activity while GANT61 targets the GLI protein aberrantly expressed in the Hh pathway, which is often active in the CSCs. This nanoformulation would be characterized for their physicochemical properties and later checked for it anti-cancer activity in breast cancer cells.

Chapter 6 compiles the “Conclusion” of Ankita’s research work, which explains the results that she achieved so far to support her claim.

【審査結果】Summary and decision

Ankita’s thesis entitled “**Therapeutic polymeric nanoparticles for targeting and destruction of cancer stem cells (CSCs)**” underlies the approach of development of a prospective strategy to target CSCs with the aid of nanotechnology focused on the synthesis of (a) Hedgehog pathway GLI Antagonist (GANT61), known to have superior anti-cancer effects over existing Hedgehog pathway inhibitors and encapsulated inside a biocompatible polymeric Nano carrier.; (b) Bio-functionalized the GANT61 nanoformulation with an aptamer to achieve active targeting in cancer cells. The targeted aptamer GANT61 nanoformulation imparted cytotoxicity towards cancer cells even at a low dose in comparison to the non-targeted GANT61 nanoformulation; and (c) To achieve complete elimination of tumor mass by killing the bulk tumor cells as well as the cancer stem cells, she further incorporated a dual drug combination therapy by encapsulating GANT61 and curcumin inside PLGA nanoparticles. 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) Ankita developed GANT61, which can modulate the Hedgehog pathway; encapsulated in PLGA biopolymer; that can effectively kill cancer stem cells.

(2) Though GANT61 proved to be highly effective to kill cancer cells; but due to its poor bioavailability based on its hydrophobicity; Anktia’s approach to encapsulate GANT61 by using PLGA proved to be a game changer and the resultant nanoformulation could kill cancer cells highly effective way.

(3) To increase further effectiveness, she incorporated A15 aptamers that can target CD133, which overexpresses on colon cancer cells. By doing so she could enhance the effectiveness in killing cancer stem cells compared to non-targeting nanoformulations.

(4) To kill tumor mass along with cancer stem cells she incorporated curcumin which an anti tumor drug in the nanoformulations. This strategy increased the effectiveness of the nanoforumation in killing the tumor mass as well as cancer stem cells. In this case the folate was used to target the cancer cells and this strategy was really increased the effectiveness in killing breast cancer cells.

The results obtained by the present doctoral study have been highly appreciated by

nanotechnologists and two first-authoring papers have been published by international journals (Oncogenesis (Nature Publishing Group) and J. Nanoworld). She presented her results in international seminars too, which received wide appreciations.

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 may well make a great contribution to the development of new methodologies for synthesising nanodrugs to kill cancer stem cells as well as tumor mass. This will clearly fetch away the possibility of relapse in the cancer therapy. The present results may also contribute to the development of advanced drug delivery methods. In conclusion, the thesis is considered to be a high quality, high standard one by international standards.