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HSA IIA subdomain-based developing anticancer metal prodrug: a new and improved approach

“It may be feasible to design a multitarget anticancer metallic prodrug based on the human serum albumin carrier to synergistically improve delivery efficiency, anticancer activity and selectivity and overcome resistance.”

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Although cisplatin is a promising anticancer agent, multifactorial resistance, serious side effects and general toxicity have limited its use in cancer chemotherapy. Therefore, less toxic and more effective metal-based anticancer compounds have been sought and developed, and drug delivery systems have been exploited to improve the selectivity of anticancer drugs and decrease their side effects [1,2].

Metallic compounds offer distinct biological and chemical diversity from that of organic drugs [3]. The biological activity of various metals in complexes with organic ligands is higher than that of the ligands alone, particularly for antitumor activity. Therefore, designing and synthesizing a metal complex derived from ligands with anticancer activity may be a strategy for developing novel anticancer metallodrugs [4]. A large number of metallic compounds have been evaluated *in vitro* and *in vivo* and some have reached clinical trials, but improving the efficacy of metallic drugs and decreasing their side effects *in vivo* remains a major challenge. Solutions to overcome these problems may lie in finding a suitable drug delivery strategy [5]. Various delivery systems, such as prodrug, ligand designed and carrier systems, have been developed to enhance the performance of these novel metallodrugs.

Human serum albumin (HSA) is the most abundant protein in plasma. HSA contains many active residues, including histidine, cysteine and lysine. Owing to the several binding sites in HSA, it can bind a diverse group of exogenous compounds [6–8]. HSA-based drug delivery systems are promising because HSA is a nontoxic, nonantigenic, biocompatible and biodegradable protein that lacks immunogenicity [9–11]. Owing to the unique properties of HSA, it can be applied as a carrier to design a metallic drug to synergistically improve anticancer activity and target metallic agents. HSA can be conjugated with a prodrug designed to contain a group that reacts with HSA special residues or an HSA complex can be formed by binding the drug to HSA [12–18].

Developing an anticancer copper(II) prodrug based on the Lys199 & His242 residues in the HSA IIA subdomain

Three potential problems are encountered when preparing HSA–drug conjugates: the excess chemical modification linkers used to tether drugs to the HSA residues can precipitate the protein; metallic prodrugs tethered to HSA dangle outside HSA, exposing them to other plasma proteins; consequently, these proteins may compete with HSA for bind-

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ing to the metallic prodrug, allowing the prodrug to escape from the HSA carrier and bind to other plasma proteins, which could cause unexpected side effects and premature release of the metallic prodrug from HSA because of weak binding or the prodrug is not released from the HSA carrier in tumor cells due to tight binding.

We propose designing an anticancer metallic prodrug based on the nature of cancer cells and the HSA carrier IIA subdomain to synergistically improve anticancer activity and targeting and delivery efficiency of metallic agents [18]. First, a metallic anticancer prodrug could be designed with leaving groups that initially bind a large hydrophobic cavity in the HSA IIA subdomain. Once the prodrug is bound, the N-donor residues (Lys199 or/and His242) of the HSA IIA subdomain would displace the metallic prodrug leaving groups and coordinate at the metal center to form a stable HSA complex. Subsequently, the HSA N-donor residues would become protonated because of the acidic environment in cancer cell lysosomes. This would decrease their ability to coordinate with the metal ion, and the metallic drug would be released from the HSA carrier. Thus, we used anticancer Cu compounds derived from the aroylhydrazone Schiff-base and breast cancer (MCF-7) cells to confirm our hypothesis.

The structures of the HSA and Cu prodrug complexes revealed that the Cu compound bound to a large hydrophobic pocket delimited by Arg218, Arg222, Lys199, Trp214, Leu219, Phe223, Leu238, His242, Arg257, Leu260, Ile264, Ser287, Ile290 and Ala291 residues [18]. Lys199 and His242 coordinate with Cu(II) by replacing the leaving group(s) of the Cu(II) compound, allowing the Cu(II) compound to strongly bind in the hydrophobic cavity of the HSA IIA subdomain. The binding affinity of the Cu compound to HSA decreased drastically in the acidic environment, and approximately 80% of the Cu compound was released from HSA [18]. Importantly, the HSA complex had a stronger anticancer capacity than the metallic compound alone because the HSA complex selectively accumulated in cancer cells to some extent [18].

Developing an anticancer metallic prodrug based on the HSA IIA subdomain Lys199 or His242 residue

Two potential problems can occur when a metallic compound is delivered to cancer cells by complexing with an HSA carrier *in vivo*. First, the metallic compound could be released prematurely from HSA because of weak binding or not released at all due to tight binding in the tumor cells. Second, because

Lys199 and His 242 would replace two groups, the metallic compound released from the HSA carrier may lose two coordination groups, which could decrease its anticancer activity relative to the original metallic compound because the coordination number of the metallic compound would affect the anticancer activity [19]. Therefore, we should regulate one potential leaving group in the metallic compound to be displaced by HSA Lys199 or His242 and prevent decreasing its anticancer activity through relationally modifying ligand in metallic compound: two groups were designed and one group had stronger coordination ability with the metal than that of Lys199/His242, which prevented this group from being replaced by Lys199/His242; the structure of the main pharmacophore in the metallic compound was modified, allowing Lys199/His242 to only replace the one potential leaving group because the binding mode of the drug to the HSA IIA subdomain was directly tied to its molecular structure.

Developing a multitarget anticancer metallic prodrug based on the HSA carrier

The majority of metallic drugs have major drawbacks, because they are efficient only for a limited number of cancers, and some tumors have acquired or intrinsic resistance, resulting in severe side effects [19]. Developing an anticancer drug targeting proteins related to cancer is promising to circumvent these drawbacks [20]. Yang *et al.* showed that Cu compounds kill cancer cells through multiple anticancer mechanisms, including promoting cell apoptosis, inhibiting the cell cycle, producing reactive oxygen species and regulating the activity of several proteins expressed in cancer cells [18]. These results suggest that cancer cells can be destroyed completely by designing an anticancer metallic drug that inhibits or kills cancer cells in several ways. The anticancer capacity of metallic compounds can be improved relative to the metallic compound alone by complexation with HSA. It may be feasible to design a multitarget anticancer metallic prodrug based on the HSA carrier to synergistically improve delivery efficiency, anticancer activity and selectivity and overcome resistance.

In conclusion, developing anticancer metal prodrug based on the nature of HSA carrier IIA subdomain may be a promising approach for synergistically enhance the selectivity, therapeutic efficacy and delivery efficiency of anticancer metal drug *in vivo*.

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