Review Article

Melanoma-Targeted Chemothermotherapy and In Situ Peptide Immunotherapy through HSP Production by Using Melanogenesis Substrate, NPrCAP, and Magnetite Nanoparticles

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Exploitation of biological properties unique to cancer cells may provide a novel approach to overcome difficult challenges to the treatment of advanced melanoma. In order to develop melanoma-targeted chemothermotherapy, a melanogenesis substrate, N-propionyl-4-S-cysteaminylphenol (NPrCAP), sulfur-amine analogue of tyrosine, was conjugated with magnetite nanoparticles. NPrCAP was exploited from melanogenesis substrates, which are expected to be selectively incorporated into melanoma cells and produce highly reactive free radicals through reacting with tyrosinase, resulting in chemotherapeutic and immunotherapeutic effects by oxidative stress and apoptotic cell death. Magnetite nanoparticles were conjugated with NPrCAP to introduce theranostic and immunotherapeutic effects through nonapoptotic cell death and generation of heat shock protein (HSP) upon exposure to alternating magnetic field (AMF). During these therapeutic processes, NPrCAP was also expected to provide melanoma-targeted drug delivery system.

1. Introduction

The incidence of melanoma is increasing worldwide at an alarming rate [1, 2]. As yet, management of metastatic melanoma is an extremely difficult challenge. Less than 10% with metastatic melanoma patients survive currently for five years because of the lack of effective therapies [3]. There is, therefore, an emerging need to
develop innovative therapies for the control of metastatic melanoma.

The major advance of drug discovery for targeted therapy to cancer cells can be achieved by exploiting their unique biological property. The biological property unique to the melanoma cell resides in the biosynthesis of melanin pigments, that is, melanogenesis occurring within specific compartments, melanosomes. Melanogenesis begins with the conversion of amino acid, tyrosine to dopa and subsequently to dopaquinone in the presence of tyrosinase. This pathway is uniquely expressed by all melanoma cells. It is well known that the clinically “amelanotic” melanoma tissues always have tyrosinase activity to some extent, and that “in vitro amelanotic” melanoma cells become “melanotic” ones when they are regrown in the in vivo condition. Melanin precursors are inherently cytotoxic through reacting with tyrosinase to form unstable quinone derivatives [4]. Thus, tyrosine analogues that are tyrosinase substrates can be good candidates for developing drugs to melanoma-targeting therapies [5].

2. Melanogenesis Substrate as a Potential Candidate for Development of Selective Drug Delivery System and Cytotoxicity to Melanoma

2.1. Synthesis of Sulfur-Amine Analogues of Tyrosine, Cysteaminylphenols, and Their Selective Incorporation into Melanogenesis Cascade. With the interaction of melanocyte-stimulating hormone (MSH)/melanocortin 1 receptor (MCIR), the melanogenesis cascade begins from activation of microphthalmia transcription factor (MITF) for induction of either eu- or pheomelanin biosynthesis. Tyrosinase is the major player in this cascade. Tyrosinase is a glycoprotein, and its glycosylation process is regulated by a number of molecular chaperons, including calnexin in the endoplasmic reticulum [24, 25]. Vesicular transport then occurs to carry tyrosinase and its related proteins (TRPs) from trans-Golgi network to melanosomal compartments, which appear to derive from early and late endosomal compartments. In this process a number of transporters, such as small GTP-binding protein, adaptor proteins, and PI3-kinase, play important roles. Once melanin biosynthesis is completed to conduct either eu- or pheomelanogenesis within melanosomes, they then move along dendritic processes and are transferred to surrounding keratinocytes in normal skin [26–28]. In metastatic melanoma cells, however, there will be practically no melanosome transfer inasmuch as there will be no receptor cells such as keratinocytes. Thus melanosomes synthesized by melanoma cells are aggregated within autophagic vacuoles in which melanogenesis-targeted drugs will be retained. In order to utilize this unique melanogenesis pathway for developing melanoma-targeted drugs, N-acetyl and N-propionyl derivatives of cysteaminyolphenols (NAc- and NPrCAPs) have been synthesized [8, 29] (Figure 1).

2.2. In Vivo and In Vitro Melanocyte Toxicity and Anti-Melanoma Effects of Cysteaminyolphenols (CAPs). Both NPrCAP and NACAP were found to selectively disintegrate follicular melanocytes after single or multiple ip administration to newborn or adult C57 black mice, respectively [12, 30]. In the case of adult mice after repeated ip administration of NPrCAP, white follicles with 100% success rate can be seen at the site where hair follicles were plucked to stimulate new melanocyte growth and to activate new tyrosinase synthesis. A single ip administration of NPrCAP into a new born mouse resulted in the development of silver follicles in the entire body coat. The selective disintegration of melanocytes which is mediated by apoptotic cell death can be seen as early as in 12 hr after a single ip administration. None of surrounding keratinocytes or fibroblasts showed such membrane degeneration and cell death [31, 32] (Figure 3).

A high, specific uptake of NAcCAP was seen in vitro by melanoma cell lines compared to nonmelanoma cells [9]. A melanoma-bearing mouse showed, on the whole body autoradiogram, the selective uptake and covalent binding of NACAP in melanoma tissues of lung and skin [6]. The specific cytotoxicity of NPrCAP and NACAP was examined on various types of culture cells by MTT assay, showing that only melanocytic cells except HeLa cells possessed the low IC50 [8, 9]. The cytotoxicity on DNA synthesis inhibition was timedependent and irreversible on melanoma cells but was transient on HeLa cells [10].

The in vitro culture and in vivo lung metastasis assays showed the melanoma growth can be blocked by administration of NACAP combined with buthionine sulfoximide (BSO), which blocked the effect of antioxidants through reducing glutathione levels. There was a marked growth inhibition of cultured melanoma cells in the presence of BSO
indicating that the selective cytotoxicity by CAP is mediated by the production of cytotoxic free radicals. The in vivo lung metastasis experiment also showed the decreased number of lung melanoma colonies [6]. The problem was, however, that a fairly large number of amelanotic melanoma lesions were seen to grow in the lung [6]. NPrCAP has been developed and conjugated with magnetite nanoparticles in the hope of increasing the cytotoxicity and overcoming the problem.

### 3. Conjugation of NPrCAP with Magnetite Nanoparticles and In Vivo Evaluation of Melanoma Growth Inhibition with/without Thermotherapy

#### 3.1. Synthesis for Conjugates of NPrCAP with Magnetite Nanoparticles and Their Selective Aggregation in Melanoma for Development of Chemo-Thermo-Immunotherapy

Magnetite nanoparticles have been employed for thermotherapy in a number of cancer treatments including human gliomas and prostate cancers [33–35]. They consist of 10–100 nm-sized iron oxide (Fe$_3$O$_4$) with a surrounding polymer coating and generate heat when exposed to AMF [12]. We expected the combination of NPrCAP and magnetite nanoparticles to be a potential source for developing not only antimelanoma pharmacologic but also immunogenic agent. Based upon the melanogenesis-targeted drug delivery system (DDS) of NPrCAP, NPrCAP/magnetite nanoparticles complex was expected to be selectively incorporated into melanoma cells. It was also hypothesized that the degradation of melanoma tissues may occur from oxidative and heat stresses by exposure of NPrCAP to tyrosinase and by exposure of magnetite nanoparticles to AMF. These two stress processes may then produce the synergistic or additive effect for generating...
tumor-infiltrating lymphocytes (TIL) by *in situ* formation of peptides that will kill melanoma cells in distant metastases (Figure 2).

In order to develop effective melanoma-targeted chemotherapy (by NPrCAP) and thermo-immunotherapy (by magnetite nanoparticles with HSP), hence providing a basis for chemo-thermo-immunotherapy (CTI therapy), we synthesized conjugates of NPrCAP and magnetite nanoparticles, on which NPrCAP is bound directly or indirectly on the surface of magnetite nanoparticles or magnetite-containing liposomes (Figure 4). Among these NPrCAP and magnetite complexes listed in Figure 4, NPrCAP/M and NPrCAP/PEG/M were chemically stable, did not lose biological property, and could be filtered as well as easily produced in large quantities. Most of the experiments described below were carried out by employing the direct conjugate of NPrCAP and magnetite nanoparticles, NPrCAP/M. A preliminary clinical trial, however, used NPrCAP/PEG/M to which polyethylene glycol (PEG) was employed to conjugate NPrCAP and magnetite nanoparticles.

In our studies, we found that NPrCAP/M nanoparticle conjugates were selectively aggregated in melanoma cells compared to non-melanoma cells [36]. The conjugates of NPrCAP and magnetite nanoparticles would be selectively aggregated on the cell surface of melanoma cells through still unknown surface receptor and then incorporated into melanoma cells by early and late endosomes. The conjugates were then incorporated into melanosomal compartment as the stage I melanosomes derive from late endosome-related organelles, to which tyrosinase was transported from the trans-Golgi network by vesicular transport [26].

### 3.2. *In Vivo* Growth Inhibition of Mouse Melanoma by Conjugates of NPrCAP and Magnetite Nanoparticles with/without Thermotherapy

The intracellular hyperthermia using magnetic nanoparticles is effective for treating certain types of primary and metastatic cancers [11, 12, 35–39]. Incorporated magnetic nanoparticles generate heat within the cells after exposure to the AMF due to hysteresis loss or relaxational loss [13, 40]. In our study of B16 melanoma cells using B16F1, B16F10, and B16OVA cells, we compared the thermo-therapeutic protocols in detail by evaluating the growth of the re-challenge melanoma transplants as well as the duration and rates of survival of melanoma-bearing mice.

By employing B16F1 and F10 cells, we first evaluated the chemotherapeutic effect of NPrCAP/M with or without AMF exposure which generates heat. NPrCAP/M without heat inhibited growth of primary transplants to the same degree as did NPrCAP/M with heat, indicating that NPrCAP/M alone has a chemotherapeutic effect. However, there was a significant difference in the melanoma growth inhibition of re-challenge transplants between the groups of NPrCAP/M with and without heat. NPrCAP/M with AMF exposure showed the most significant growth inhibition in re-challenge melanoma transplants and increased life span of the host animals, that is, almost complete rejection of re-challenge melanoma growth, whereas NPrCAP/M without heat was much less, indicating that NPrCAP/M with heat possesses a thermo-immunotherapeutic effect (Figures 5(a), 5(b), and 5(c)).

Specifically our study indicated that the most effective thermo-immunotherapy for re-challenge B16F1 and F10 melanoma cells can be obtained at a temperature of 43°C for 30 min with the treatment repeated three times on every other day intervals without complete degradation of the primary melanoma [37]. This therapeutic approach and its biologic effects differ from those of magnetically mediated hyperthermia on the transplanted melanomas reported previously. In previous studies by Suzuki et al. [38] and Yanase et al. [39], cationic magnetoliposomes were used for B16 melanoma. They showed that hyperthermia at 46°C once or twice led to regression of 40–90% of primary tumors and to 30–60% survival of mice, whereas their hyperthermia at 43°C failed to induce regression of the secondary tumors and any increase of survival in mice [38, 39].

### 4. Production of Heat Shock Protein, Nonapoptotic Cell Death, and Tumor-Infiltrating Lymphocytes by Conjugates of NPrCAP and Magnetite Nanoparticles with Thermotherapy

#### 4.1. Production of Heat Shock Protein and Non-Apoptotic Melanoma Cell Death by NPrCAP/Magnetite Nanoparticle Conjugates with Thermotherapy

It has been shown that hyperthermia treatment using magnetite cationic liposomes (MCLs), which are cationic liposomes containing 10-nm magnetite nanoparticles, induced antitumor immunity through HSP expression [12, 22, 41, 42]. In our studies using B16F1, F10, and OVA melanoma cells [43], the hyperthermia using NPrCAP/M with AMF exposure also showed antitumor immune responses via HSP-chaperoned antigen (Figure 6) [43]. It may be speculated that the HSPs-antigen peptide complex released from melanoma cells treated with this intracellular hyperthermia is taken up by dendritic cells (DCs) and cross-presented HSP-chaperoned peptide in the context of MHC class I molecules [44]. In our CTI therapy with AMF exposure, the heat-mediated melanoma cell necrosis was induced to NPrCAP/M-incorporated cells. In this group, we also found that repeated hyperthermia (3 cycles of NPrCAP/M administration and AMF irradiation) was required to induce the maximal antitumor immune response [37].

If melanoma cells escaped from this necrotic cell death, repeated hyperthermia should produce further necrotic cell death to the previously heat-shocked melanoma cells in which HSPs were induced. Our CTI therapy with AMF exposure using B16OVA cells showed that Hsp72/Hsc73, Hsp90, and ER-resident HSPs participated in the induction of CD8+ T-cell response [43]. Different from the results of B16F1 and F10 cells, Hsp72 was largely responsible for the augmented antigen presentation to CD8+ T cells. As Hsp72 is known to upregulate in response to hyperthermia or heat shock treatment [41], newly synthesized Hsp72 has a chance to bind to the heat-denatured melanoma-associated antigen.
Figure 3: Depigmenting effect of NPrCAP. (a) Depigmentation of C57 black mouse hair follicles by a single ip administration of NPrCAP or NAcCAP results in complete loss of melanin pigmentation. Entire coat color changes to silver from black. Electron microscopic observation reveals selective degradation of melanocytes and melanogenic organelles such as early-stage melanosomes at 6 hr after administration. At 24 hr after administration, these melanocytes reveal total degradation. (b) Depigmentation of black skin after topical application of NPrCAP. There is a marked decrease of melanocyte populations after topical application. Electron microscopic observation indicates selective accumulation of NPrCAP in the tyrosinase areas such as in melanosomes and Golgi apparatus as indicated by the deposition of electron dense materials (see arrows).

Figure 4: Conjugates of NPrCAP/magnetite nanoparticles for developing melanogenesis-targeted melanoma nanomedicine.
Figure 5: Melanoma growth and survival of melanoma-bearing mice by CTI therapy using NPrCAP/M with and without AMF exposure. (a) Experimental protocols. (b) Tumor volumes of rechallenge B16F1 melanoma transplants. (c) Kaplan-Meier survival of melanoma-bearing mice after treatment following experimental protocols of Figure 5(a).
CD8+ T cells in inguinal DLNs increased significantly in the mice treated with NPrCAP/M-mediated hyperthermia.

5. Melanocytotoxic and Immunogenic Properties of NPrCAP without Hyperthermia

5.1. Induction of Apoptosis, Reactive Oxygen Species (ROS), and Tumor-Specific Immune Response by NPrCAP Administration Alone. In our animal study, those animals bearing B16F1 and B16F10 melanoma cells showed, to certain degree, rejection of second re-challenge melanoma transplantation by administration of both NPrCAP alone and NPrCAP/M minus AMF exposure [46]. Our working hypothesis for this finding is that there is a difference in the cytotoxic mechanism and immunogenic property of NPrCAP/M between experimental groups with and without hyperthermia by AMF exposure. The animals with NPrCAP/M without AMF exposure resulted in non-necrotic, apoptotic cell death. The animals with NPrCAP/M plus AMF exposure, on the other hand, resulted in nonapoptotic, necrotic cell death with immune complex production of melanoma peptide as well as Hsp70 and a small amount of Hsp 90.

To further examine the mechanism of the cell death induced by NPrCAP, those cells treated with NPrCAP alone were subjected to flow cytometric analysis, caspase 3 assay, and TUNEL staining [46]. The sub-G1 fraction was increased in the NPrCAP-treated B16F1 cells, comparable to TRAIL-exposed B16F1, but not in the NPrCAP-treated non-melanoma cells (NIH3T3, RMA) or nonpigmented melanoma cells (TXM18) (Figure 7). The luminescent assay detected caspase 3/7 activity in the NPrCAP-treated B16F1 cells remarkably increased (35.8-fold) compared to that in the nontreated cells. NIH3T3, RMA, and TXM18 cells treated with TRAIL showed 10.6-, 71.1-, and 5.8-fold increases of caspase 3/7 activation compared to the control, respectively, whereas those with NPr-4-S-CAP showed increases of 4.1-, 1.4-, and 1.8-fold, respectively. The number of TUNEL-positive cells was significantly increased only in the B16F1 tumor treated with NPrCAP. This increase was not observed in the B16F1 tumor without NPrCAP or in the RMA tumors with or without NPrCAP. The findings indicate that NPrCAP induces apoptotic cell death selectively in melanoma cells.

5.2. Melanocytotoxic and Immunogenic Properties of NPrCAP Compared to Monobenzyl Ether Hydroquinone. Monobenzyl ether of hydroquinone has long been known to produce the skin depigmentation at both the drug-applied area by direct chemical reaction with tyrosinase and the non-applied distant area by immune reaction with still unknown mechanism [43, 48–50]. The melanogenesis-related cytotoxicity primarily derives from tyrosinase-mediated formation of dopaquinone and other quinone intermediates, which produce ROSs such as superoxide and H2O2 [4, 31, 32, 51]. This unique biological property of melanin intermediates not only causes cell death, but also may produce immunogenic properties. We postulated that the cytotoxic action of NPrCAP appears to involve two major biological processes. One is
cytostatic process which derives from the DNA synthesis inhibition through the interaction of quinone and free radicals with SH enzymes and thymidine synthase. Another is the cytocidal process by damage of DNA and mitochondrial ATP through oxidative stress and interaction with SH-enzyme [10]. They bind protein disulphide isomerase [32].

Monobenzyl ether form of hydroquinone was shown to produce a reactive ortho-quinone generated by tyrosinase-catalyzed oxidation and self-coupling and thiol conjugation reactions [53]. It was also shown to induce cell death without activating the caspase cascade or DNA fragmentation, indicating that the death pathway is non-apoptotic [53, 54]. It was further suggested that monobenzyl ether hydroquinone induced the immunogenicity to melanocytes and melanoma cells by forming quinone-hapten to tyrosinase and protein antigen recognized by T cells-1 (MART-1) containing CD8+ T-cells. The latter cells kill monobenzone-exposed melanocytes expressing haptenated antigens, in turn, trigger an immunological response cascade that results in a melanocyte-specific delayed-type hypersensitivity reaction leading to melanocyte elimination to produce depigmentation in vitiligo and melanoma rejection. We examined the tyrosinase-mediated oxidation of NPrCAP and its subsequent binding to sulphhydryl compounds (thiols) in NPrCAP-treated melanoma tissues and demonstrated that NPrCAP is oxidized by tyrosinase to form a highly reactive ortho-quinone, (N-propionyl-4-S-cysteaminylcatechol, NPrCAQ; Figure 8), which then binds covalently to biologically relevant thiols including proteins through the cysteine residues. In vitro and in vivo studies were also conducted to prove the binding of the quinone-hapten NPrCAQ to proteins. The thiol adducts were analyzed after acid hydrolysis as 5-S-cysteaminyl-3-S-cysteinylicatechol (CA-CysC) (Figure 8). Our results specifically provided evidence that NPrCAP is oxidized by tyrosinase to an ortho-quinone, NPrCAQ, which is highly reactive yet stable enough to survive and then interact with biologically relevant thiols to form covalent adducts. The activation of NPrCAP to NPrCAQ by tyrosinase and the subsequent binding to proteins through cysteine residues were also demonstrated in the in vitro and in vivo experiments. Our finding was the first demonstration that the quinone-protein adduct formation actually takes place in melanoma cells and melanoma tissues through the tyrosinase-mediated mechanism. Furthermore, 60–80% of the NPrCAQ-thiol adducts were found in the protein fraction in melanoma cells and in the tumors. This is surprising when we consider the much lower reactivity of protein sulphhydryl groups compared with those in small thiols such as cysteine [60, 61]. The remaining nonprotein SH adducts were produced by the reaction of NPrCAQ with free cysteine or glutathione as a detoxifying mechanism. In this connection, it was previously shown that the depletion of glutathione augmented the melanocytotoxicity and antime-lanoma effects of NAcCAP [62].

According to the potent melanoma immunotherapy theory using monobenzene [54, 55, 57–59], tyrosinase appears to trigger melanoma regression. Tyrosinase oxidation of monobenzene produces a highly reactive quinone-hapten [44, 54] and ROS concurrently [54]. The quinone-hapten binds to cysteine residues in tyrosinase or other melanoma proteins thereby generating possible neoantigen, which activate hapten-reactive CD8+ T-cells. The latter cells kill monobenzene-exposed melanocytes expressing haptenated antigens.
Figure 8: Tyrosinase activation of NPrCAP (prohapten) and binding of the quinone-hapten NPrCAQ with proteins thorough cysteine residues. Oxidation of NPrCAP with tyrosinase produces the quinone NPrCAQ, which is reduced to the catechol NPrCAC or binds to thiols (cysteine, glutathione, melanosomal proteins). The production of NPrCAQ-thiol adducts can be confirmed by the detection of CA-CysC after acid hydrolysis. NAcCys-NPrCAC is produced by the addition reaction of NAcCys (R-SH) with NPrCAQ. From Ito et al. [47].

Figure 9: Scheme of intracellular hyperthermia using NPrCAP/PEG/M or NPrCAP/M with AMF exposure. NPrCAP/PEG/M nanoparticles are selectively incorporated in melanoma cells. Intracellular hyperthermia can induce necrotic cell death, and adjacent live melanoma cells suffer heat shock, resulting in increased level of intracellular HSP-peptide complexes. Repeated hyperthermia turns heat-shocked cells to necrotic cells, leading to the release of HSP-peptide complexes into extracellular milieu. The released HSPs-peptide complexes are taken up by dendritic cells (DCs). Then, DCs migrate into regional lymph nodes and cross-present HSP chaperoned antigenic peptides to CD8+ T-cell context of MHC class I molecules, thereby inducing antimelanoma cytotoxic CD8+ T-cells.

6. Summary and Conclusion

Several clinical trials using melanoma peptides or an antibody that blocks cytotoxic T-lymphocyte-associated antigen on lymphocytes have been shown to improve overall melanoma survival [64–66]. Promising oncogene-targeted melanoma therapy has also been successfully introduced recently [67].

Our study may however indicate that exploitation of a specific biological property to cancer cells can be another approach for developing novel melanoma-targeted drugs which can also trigger the production of melanoma-targeted in situ vaccine. Our approach using melanogenesis substrate and magnetite nanoparticles is based upon the expectation of (i) direct killing of melanoma cells by chemotherapeutic and thermo-therapeutic effect of melanogenesis-targeted drug (NPrCAP/M) and (ii) indirect killing by immune reaction (in situ peptide vaccine) after exposure to AMF. It is hoped from these rationales that a tumor-specific DDS is developed by NPrCAP, and selective cell death can be achieved by exposure of conjugates of NPrCAP/M nanoparticles to AMF. Hyperthermia increases the expression of intracellular HSPs which leading to the presentation of melanosome-derived antigens and the induction of antigen-specific T-cell responses [58].

These immunological events can also be expected to occur for our NPrCAP because the involvement of CD8+ T cells and the production of ROS in NPrCAP-treated melanoma cells were demonstrated in our previous study [46]. We expect the production of NPrCAC through redox exchange in melanoma cells and the subsequent production of ROS from the catechol because the closely related catechol, 4-S-cysteaminylcatechol, was shown to produce superoxide radicals (which are rapidly converted to hydrogen peroxide) [63]. The thiol adduct RS-NPrCAC, as a catechol, may also contribute to the production of ROS.

antigens on their surface, further liberating melanocyte antigens for presentation by dendritic cells. Finally, the antigen-specific T-cell response is induced and propagated [54, 57–59]. The ROS generated also causes damage to melanosomes

leading to the presentation of melanosome-derived antigens and the induction of antigen-specific T-cell responses [58].

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is important in and necessary for the induction of antitumor immunity [41, 68]. Overexpression of HSPs increases tumor immunogenicity by augmenting the chaperoning ability of antigenic peptides and presentation of antigenic peptides in MHC class I molecules [39, 69]. In this process professional antigen-presenting dendritic cells play unique and important roles in taking up, processing, and presenting exogenous antigens in association with MHC class I molecules. Our study indicated that combination of melanogenensis substrate, NPrCaP, and local magnetite nanoparticles with hyperthermia could induce in situ a form of vaccine against tumor cells and may be effective not only for primary melanoma but also for distant secondary metastases (Figure 9).

Interestingly we found that NPrCaP by itself has potent chemotherapeutic and immune-adjuvant effects. It was demonstrated that the phenol NPrCaP, as a prohapten, can be activated in melanoma cells by tyrosinase to the reactive quinone-hapten NPrCaQ which binds to melanosomal proteins through their cysteine residues to form possible neo-antigens, thus triggering the immunological response (Figure 8).

### Abbreviations

- AMF: Alternating magnetic field
- BSA: Bovine serum albumin
- BSO: Buthionine sulfoximide
- CA-CysC: 5-S-cysteaminyl-3-S-cysteinylcatechol
- CAP: Cysteaminylnophenol
- CDR3: Third complementarity determining region
- CML: Cationic magneito-liposome
- CTI therapy: Chemothermoimmunotherapy
- CTL: Cytotoxic T lymphocyte
- DCs: Dendritic cells
- DDS: Drug delivery system
- DLNs: Draining lymph nodes
- HSP/Hsp: Heat shock protein
- IL: Interleukin
- M: Magnetite nanoparticle
- mAb: Monoclonal antibody
- MART-1: Melanoma antigen recognized by T cells-1
- MCLs: Magnetite cationic liposomes
- MCR: Melanocortin 1 receptor
- MHC: Major histocompatibility complex
- MITF: Microphthalmia transcription factor
- ML: Noncaticonic magnetoliposome
- MSH: Melanocyte stimulating hormone
- NAcCAP: N-acetyl-4-S-cysteaminylnophenol
- NDLN: Nondraining lymph node
- NPrCACP: N-proponionyl-4-S-cysteaminylnophenol
- NPrCAP: N-propionyl-4-S-cysteaminylnophenol
- NPrCaQ: N-propionyl-4-S-cysteaminylnquinine
- Nrf2: NF-E2-related factor 2
- OVA: Ovu-albumin
- PEG: Polyethylene glycol
- ROS: Reactive oxygen species
- RT-PCR: Reverse transcription polymerase chain reaction

TILs: Tumor-infiltrating lymphocytes
- TCRs: T-cell receptors

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