our previous results, the survival prolongation effects of swine skin allograft were slightly weaker than in rat model. Also, the immunosuppressive effects of β-SQAG9 were slightly weaker than FK506 actions. However, these current results implied that β-SQAG9 could prolong swine skin allograft survival in vivo (Table 1).

Immunohistochemistry of graft area

To investigate that which subset of T cells invaded the graft area relating to the allograft responses, we examined T cells invading the graft area by immunohistochemistry. There was no significant difference in the number of CD8 T cells in the graft area between the three groups (figure 3), but numbers of CD4 cells in the FK506 and β-SQAG9 group were approximately half of those in the control group (figure 4). Since FK506 is well known that T cell proliferation is strongly inhibited by suppression of IL-2 secretion in vitro and in vivo (8), the decrease of the CD4 T cell population in the current study could explain the results obtained by FK 506. In contrast, because β-SQAG9 does not possess inhibitory effects on cell proliferation (5-7), this drug might have interfered with the invasion of CD4 T cell into this area.

It is well known that exogenous antigen in allografts is initially presented to naive CD4 T cells exclusively by dendritic cells (DCs) within the T cell areas of secondary lymphoid

tissues (9). A major characteristic of naive CD4 T cell is expression of CD62L molecules that play an important role in migration between blood and lymph nodes (10-12). It was reported that $CD4^+$ T cells play a more essential role in the initial alloreaction than $CD8^+$ T cells (13). Thus CD4 T cells expressing CD62L molecules could play an important role in acute rejection. It is therefore reasonable to postulate that β-SQAG9 might inhibit migration of naïve CD4 T cells to the graft area via blockade of CD62L molecules, resulting in prolongation of graft survival. Since we could not obtain anti-swine CD62L (L-selectin) antibody, it was not clear whether or not the CD4 T cells invading the allograft area expressed CD62L molecules. Further investigations should prove interesting.

In conclusion, β-SQAG9 showed immunosuppressive effect in swine allogeneic skin transplantation. These data suggested that β-SQAG9 has promising potential as an immunosuppressant.

Acknowledgements

This work was supported by a Grant-in-Aid for New Energy and Industrial Technology Development Organization (NEDO) of Japan.

References

1. Chiba K, Yanagawa Y, Masubuchi Y, et al. FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rat. I. FTY720 selectively decreases the number of circulating mature lymphocytes by acceleration of lymphocyte homing. J Immunol 1998;160:5037-44.
2. Chiba K, Hoshino Y, Suzuki C, et al. FTY720, novel immunosuppressant possessing unique mechanisms. I. Prolongation of skin allograft survival and synergistic effect in combination with cyclosporine in rat. Transplant Proc 1996; 28:1056-9.
3. Hoshino Y, Suzuki C, Ohtsuki M et al. FTY720, novel immunosuppressant possessing unique mechanisms. II. Long-term graft survival induction in rat heterotopic cardiac allograft and synergistic effect in combination with cyclosporine A. Transplant Proc 1996; 28:1060-1.
4. Kawaguchi T, Hoshino Y, Rahman F, et al. FTY720, novel immunosuppressant possessing unique mechanisms. III. Synergistic prolongation of canine renal allograft survival in combination with cyclosporine A. Transplant Proc 1996; 28:1062-3.
5. Matsumoto Y, Sahara H, Fujita T, et al. An immunosuppressive effect by synthetic sufonolipids deduced from sulfonoquinovosyl diacylglycerols of sea urchin. Transplantation 2002; 74:261-7.
6. Takenouchi M, Sahara H, Yamamoto Y, et al. The mechanism of immunosuppressive

effect in vivo of novel immunosuppressive drug, β-SQAG9 that inhibits the response of CD62L+ T cell subset. Transplant Proc 2005; 37:139-42.

7. Yamamoto Y, Sahara H, Takenouchi M, et al. Inhibition of CD62L+ T cell response in vitro via a novel sulfo-glycolipid, β-SQAG9 liposome that binds to CD62L molecule on the cell surface. Cellular Immunol 2004; 232:105-15.
8. O'Keefe SJ, Tamura J, Kincaid RL, et al. FK-506- and CsA-sensitive activation of the interleukin-2 promoter by calcineurin. Nature 1992; 357:692-4.
9. Jenkins MK, Khoruts A, Ingulli E, et al. In vivo activation of antigen-specific CD4 T cells. Annu Rev Immunol 2001; 19:23-45.
10. Richard LH, Thaker V, Pavlick MV, et al. L-selectin (CD62L) expression distinguishes small resting memory $CD4^+$ T cells that preferentially respond to recall antigen. J Immunol 2003; 170:28-32.
11. Steeber DA, Green NE, Sato S, Tedder TF Lymphocyte migration in L-selectin-deficient mice. Altered subset migration and aging of the immune system. J Immunol 1996;157:1096-106.
12. Catalina MD, Carroll MC, Arizpe H, et al. The route of antigen entry determines the requirement for L-selectin during immune responses. J Exp Med 1996;184:2341-51.
13. Butcher E, Picker L Lymphocyte homing and homeostasis. Science 1996;272:60-6.

Figure legends

Figure 1. Suppressive effects of T cell responses by β-SQAG9 on swine MLR.

T cells derived from swine recipient's PBMCs were co-cultured with APCs that gamma ray irradiated PBMCs from allogeneic donor in the presence of 1 μg/ml FK506 or β-SQAG9 at indicated concentration. After cultivation, T cells incorporated with [^{3}H]thymidine were harvested, and their radioactivity (cpm) was counted. Each bar represents the mean + SEM of triplicate determinations. Results represent one of three independent experiments. *P<0.01.

Figure 2. Gross and histological examination of grafted skin of control, FK506, and β-SQAG9 treated recipient. After transplantation, FK506 or β-SQAG9 was administered daily in vein at concentration of 0.1mg/kg or 50mg/kg, respectively, for 7 day. The control group was administered serine as a same manner. Biopsies were performed at the 7 day after skin grafting. a-c were represented gross characters at 7 day grafting. d-f were represented HE-staining tissues (x 200).

Figure 3. Immunohistochemical examination. Frozen specimens were stained with anti-

porcine CD8α chain mAb and were examined by light microscope (a-c, x 100). Arrow indicates typically the $CD8^+$ T cell. d each bar represents the mean of $CD8^+$ T cell + SEM.

Figure 4. Immunohistochemical examination. Frozen specimens were stained with anti-porcine CD4α chain mAb and were examined by light microscope (a-c, x 100). Arrow indicates typically the $CD4^+$ T cell. d each bar represents the mean $CD4^+$ T cell + SEM. $*P<0.01$.

Table 1

Result of swine skin allograft survival

Histological grade	Control	FK506	β-SQAG9
Grade 1	0/3	1/3	0/3
Grade 2	0/3	2/3	3/3
Grade3	3/3	0/3	0/3

Table shows number of grafts/number of total swine.

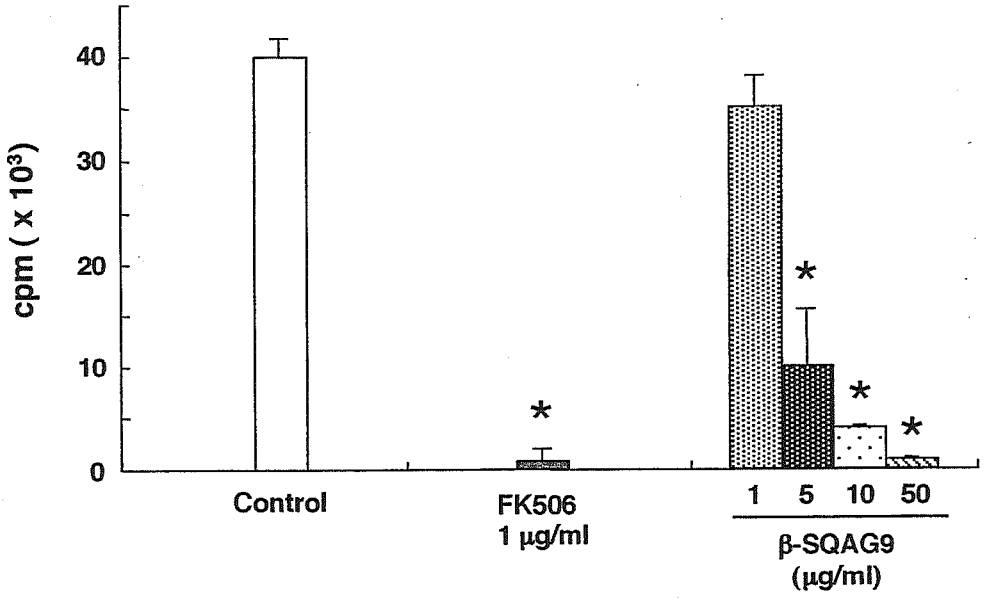


Fig. 1

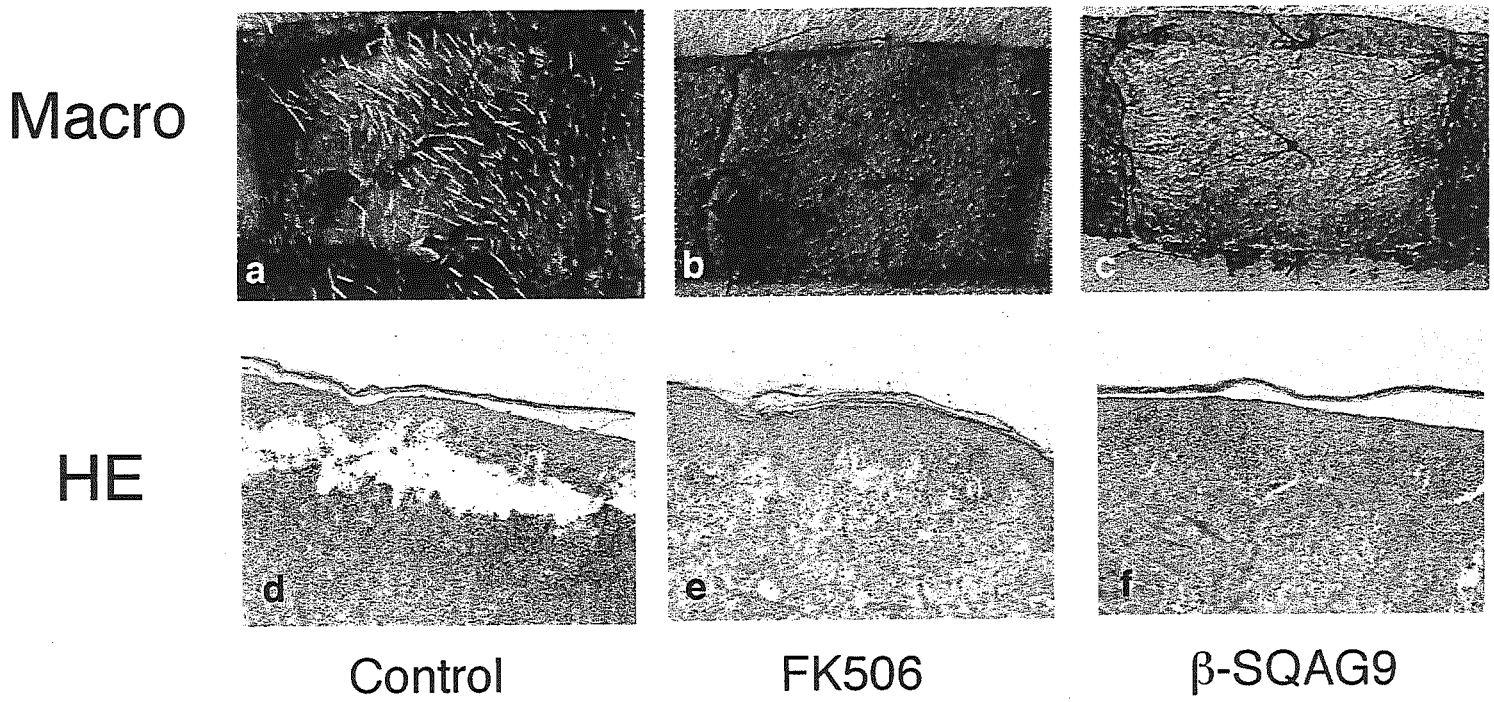
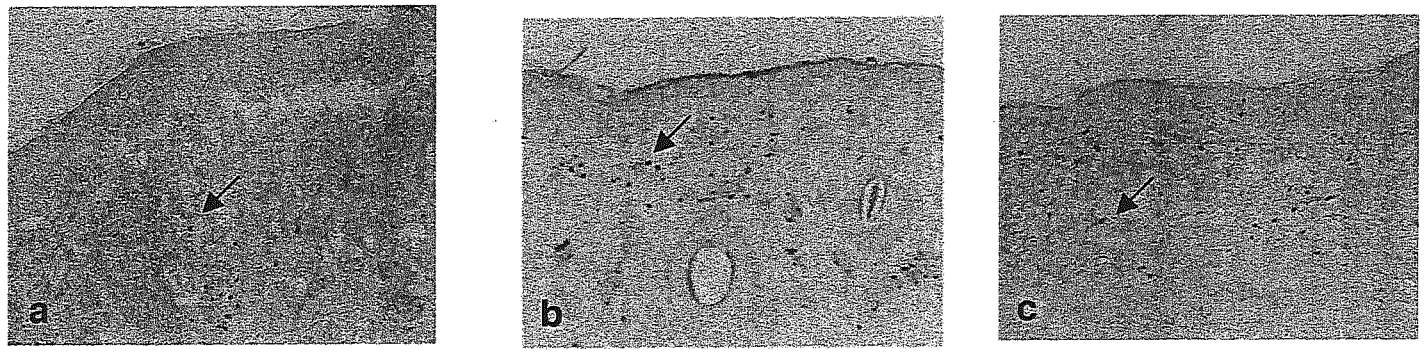


Fig. 2


a
b
c
Control
FK506
β-SQAG9

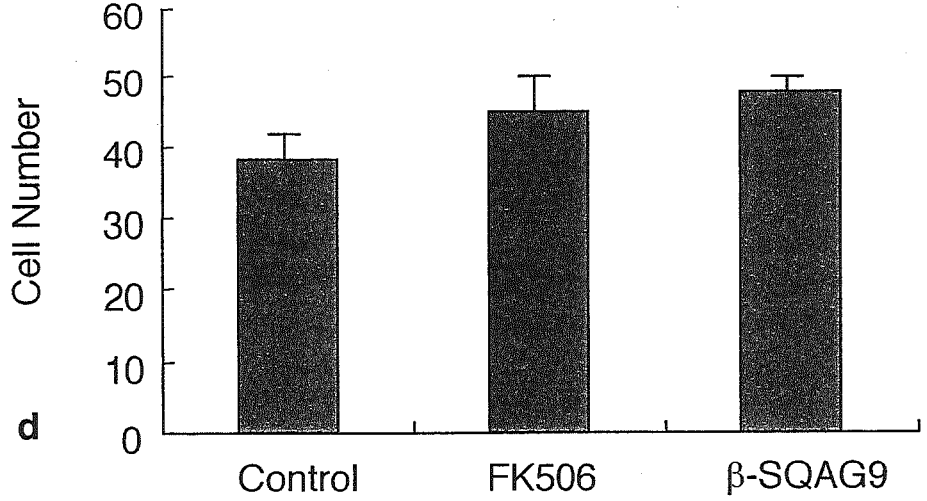
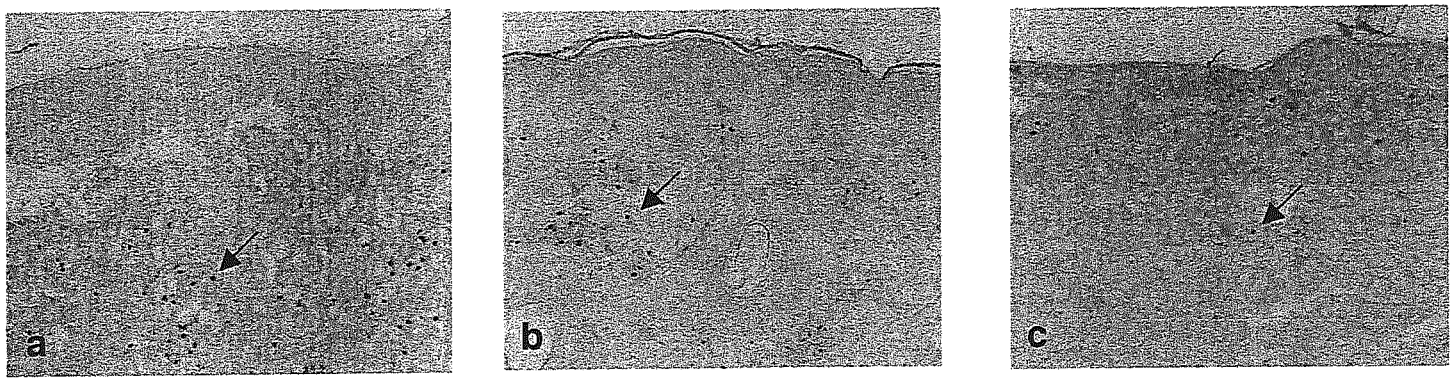

d
Cell Number
60
50
40
30
20
10
0
Control
FK506
β-SQAG9

Fig. 3


a
b
c
Control
FK506
β-SQAG9

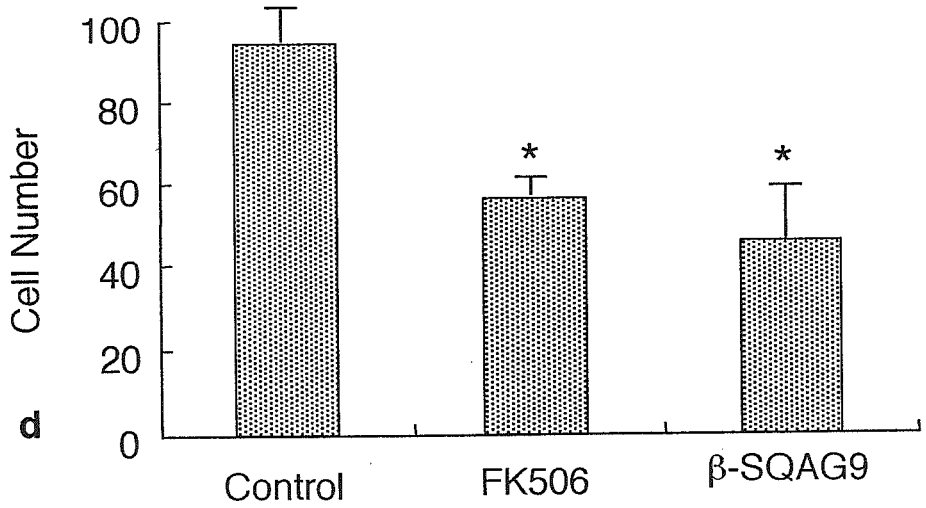

d
Cell Number
100
80
60
40
20
0
*
*
Control
FK506
β-SQAG9

Fig. 4

Melanogenesis--targeted drug delivery and chemotherapy system for development of melanoma thermo-chemotherapy using NPrCAP-magnetite nano-particles.

K Jimbow[1], T Takada[1], T Yamashita[1], M Sato[1], A Sakemoto[1], H Matsusaka[1], I Ono[1], Y Tamura[2], N Sato[2], A Ito[3], H Honda[3], K Wakamatsu[4], and S Ito[4]

[1]Department of Dermatology; [2]Department of Pathology 1, Sapporo Medical University School of Medicine, Sapporo; [3]Bioprocess Engineering Laboratory, Department of Biotechnology School of Engineering, Nagoya University, Nagoya; [4]Department of Chemistry, Fujita Health University, Toyoake, Japan.

Management of malignant melanoma with distant metastases is a difficult challenge. An effective therapeutic approach can be established if the biological property unique to melanoma cells is exploited. In our previous reports we have shown that N-acetyl or N-propionyl derivative of cysteaminyl phenol (NAcCAP or NPrCAP) is a good substrate for tyrosinase, and selectively incorporated into melanoma tissues and inhibit melanoma cell growth in *in vivo* and *in vitro* systems. In addition, we have shown that magnetites encapsulated into cationic liposomes (CML), when given intralesionally and exposed to alternating magnetic field (AMF), can inhibit melanoma growth by direct killing of melanoma cells and simulate immune reaction. However, selective incorporation of CML into melanoma tissues was shown to have limitations. In our present *in vitro* and *in vivo* study we have utilized NPrCAP as a selective drug delivery system (DDS) and anti-melanoma agent, and investigated the selectivity/specificity of NPrCAP DDS and the anti-melanoma effects by combination of NPrCAP and magnetite particles (M) or magnetite liposome complex (ML). Selective melanoma-cytocidal effect was produced by NPrCAP which was either encapsulated in non-cationic magnetite-liposome complex (NPrCAP/ML) or directly conjugated to magnetites (NPrCAP/M). In the *in vitro* culture cells, we found that ML often forms non-specific aggregation of NPrCAP to non-melanoma cells, probably through the interaction between liposomes and cell membrane lipoproteins. However, this non-specific aggregation was overcome by introduction of NPrCAP/M, which resulted in a selective uptake by melanoma cells compared to non-melanoma cells. Exposure to AMF generated a steady and immediate increase of the heat up to 46° C in the *in vivo* system, which was found to cause non-apoptotic death of melanoma cells in the *in vitro* system. This was more efficiently produced by NPrCAP/M compared to NPrCAP/ML. Daily administration of NPrCAP/M three times without AMF caused a statistically significant inhibition of growth of melanoma cells (B16F1) grown *s.c.* in C57BL mice compared to control non-treatment groups. Importantly, repeated exposures to AMF in the same experimental group produced a steady generation of heat and complete abolishment of melanoma tissues inoculated *s.c.* into mice. Thus our NPrCAP/M provided a firm basis for developing a novel, melanogenesis-targeted DDS and thermo-chemotherapy by AMF generated heat for malignant melanoma.

ナノ微粒子・チロシン（フェノール）誘導体によるメラノーマ標的療法の開発

札幌医科大学医学部皮膚科学講座　神保孝一

メラノーマは全身の皮膚、粘膜のみならず、眼、脳、脊髄等の臓器に発生する癌である。本症の発生は近年加速度的に上昇しているが、予後は極めて悪く、病初期から皮膚、リンパ節、肺、肝、脳転移をとる。外科的切除以外、有効な治療法は無い。

我々はメラノーマに特異な酵素チロシナーゼの基質でありメラノーマ細胞と特異的親和性を有するアミノ酸チロシン（フェノール）誘導体をメラノーマ標的ドラッグ・デリバリー剤であり且つ化学療法剤として用い、これを磁気ナノ微粒子（マグネタイト）と直接重合させるかリポソームに内包させマグネタイト・リポソームを形成させるかして新しいメラノーマ・ナノメディシン療法を開発する研究を行っている。マグネタイトは磁場照射により温熱を発しこの効果により熱ショック蛋白90(HSP90)産生による腫瘍免疫効果を発現する。

従い、マグネタイトの持つ温熱免疫効果とチロシン（フェノール）誘導体の持つ細胞殺効果を発現させる事により従来のメラノーマ治療法の概念とはまったく異なる新しい化学・温熱免疫ナノ・メデシンを確立することができる。しかもアミノ酸チロシン（フェノール）誘導体を磁気ナノ微粒子マグネタイトに固定化する事で、大量合成が容易で臨床応用に最も近い安定で安全な薬剤を合成する事が可能となる。

PA3-1241 RETトランスジェニックマウスに発生したメラノーマに対する磁性微粒子を用いた温熱療法の治療効果

井藤 彰[1]、中原 陽子[1]、藤岡 正剛[1]、小林 猛[1]、武田 湖州恵[2]、中島 泉[2]、本多 裕之[1]（[1]名大・院工・化生、[2]名大・院医・免疫）

The effect of hyperthermia using magnetite cationic liposomes on hereditary melanoma in RET transgenic mouse

Akira Ito[1], Yoko Nakahara[1], Masatake Fujioka[1], Takeshi Kobayashi[1], Kozue Takeda [2], Izumi Nakashima[2], Hiroyuki Honda[1] ([1]Dept. Biotechnol. School of Eng. Nagoya Univ., [2]Dept. of Immunol. Nagoya Univ. School of Med.)
E-mail: akira@nubio.nagoya-u.ac.jp

【目的】本研究室では腫瘍を特異的に加温するために、Magnetite Cationic Liposome(MCL)を用いた温熱療法を行ってきた。MCLは磁性微粒子のマグネタイトをカチオン性のリポソームで包むことで腫瘍への取り込まれやすくしたもので、これを腫瘍局所に注入し、交番磁場をかけることで、腫瘍特異的な温熱療法を行うことが可能である。本研究では、実際の患者の癌に近いモデルとして、マウスメラノーマ遺伝性発症モデルにおける抗腫瘍効果を調べた。【方法】メラノーマ遺伝性発症モデルとして、RETトランスジェニックマウスを用いた。腫瘍径が7mmに達したマウスの腫瘍局所にMCLを投与し、磁場照射を3日連続（1クール）で行った。治療部位の腫瘍が再増殖した場合は、新たにMCLを注入して磁場照射を繰り返した。【結果】MCLを用いることで45℃, 30分の温熱治療を行うことができた。1クールの治療で完全治癒しなかったマウスには繰り返し治療を複数回行うことで、5匹全てのマウスで完全退縮することに成功した。これらの結果から、MCLを用いた温熱療法は、複数回繰り返し治療を行うことで非常に効果の高い治療法になると考えられる。

Keyword: Hyperthermia, Melanoma

未熟樹状細胞を用いたHeat Immunotherapyの開発

井藤　彰[1]、田中功二[1]、小林　猛[2]、川村龍吉[3]、島田眞路[3]、松本和彦[4]、斎田俊明[4]、本多裕之[1]
[1]名古屋大院・工・化生、[2]中部大・応生、[3]山梨大・医・皮膚科、[4]信州大・医・皮膚科

Heat Immunotherapy using immature dendritic cells

Akira ITO[1], Kouji TANAKA[1], Takeshi KOBAYASHI[2], Tatsuyoshi KAWAMURA[3], Shinji SHIMADA[3], Kazuhiko MATSUMOTO[4], Toshiaki SAIDA[4], Hiroyuki HONDA[1]
[1]Dept. of Biotechnol., Sch. of Eng., Nagoya Univ., [2]Dept. of Biol. Chem., Sch. of Biosci. Biotechnol., Chubu Univ., [3]Dept. of Dermatol., Yamanashi Univ. Med. Sch., [4]Dept. of Dermatol., Shinshu Univ. Med. Sch.

【目的】本研究室では腫瘍組織を特異的に加温するためにMagnetite Cationic liposome (MCL)を用いた温熱療法を行ってきた。このMCLは、磁性微粒子のマグネタイトをカチオニックリポソームで包むことで腫瘍組織に取り込まれやすくしたもので、これを腫瘍組織局所に注入し、交番磁場を照射することで、腫瘍特異的に加温することができる。我々は、この温熱療法で腫瘍部において免疫担当細胞が集まってきていることを観察し、抗腫瘍免疫が誘導されることを見出した。この抗腫瘍免疫において、熱ショックタンパク質が重要な役割を果していることがわかっている。そこで、その役割を効果的に行なわせるために専門的抗原提示細胞の中で最も提示作用の強いといわれる樹状細胞（DC）添加治療を温熱療法と組み合わせた。

【方法】腫瘍細胞として、B16 melanomaとEL4 T lymphomaを用い、実験動物はC57/BL6 mouseを用いた。これらにより担癌マウスを作製し、腫瘍サイズが7mmに達したマウスについて、その腫瘍局所にMCLを投与し、温熱治療を行った。さらに、未熟DC (10^6細胞)を腫瘍部局所に投与し、その後の治療効果と免疫の増強について調べた。また、腫瘍に接種した未熟DCの免疫器官への遊走性と、温熱した腫瘍細胞のパルス刺激によるDCの成熟を確認した。

【結果】温熱+DC治療を施すことで、B16 melanomaにおいて10匹中6匹、EL4 T lymphomaにおいて8匹中6匹で原発の腫瘍の完全退縮に成功した。生存率が有意に伸び、治癒したCured mouseはRechallengeにおいてもすべての腫瘍を拒絶した。また、細胞障害性の測定により、温熱+DC治療によってCTLによる腫瘍特異的免疫とNK細胞による非特異的免疫の両方の活性化効果があることが確認された。これらの結果より、温熱療法と未熟DCによる免疫細胞療法の併用は効果的であると考えられる。さらに、未熟DCに温熱後の腫瘍細胞（B16）をパルス刺激することで、DCの成熟マーカーの発現が高まっていることがわかった。また、*In vivo*で腫瘍投与後の未熟DCのmigration assayによりDCリンパ節や脾臓といった免疫器官に遊走していることが確認され、未熟DCが温熱後の腫瘍に投与されて機能していることが示唆された。

Exploitation of Melanogenesis for Melanoma Control

Kowichi JIMBOW

It is well known that melanin biosynthesis is uniquely expressed in melanoma cells and if overproduced, toxic and kill them. Our approach to control melanoma growth is based upon the exploitation of potentially toxic melanin biosynthesis pathway by developing sulphur-containing derivatives of tyrosine, a natural tyrosinase substrate. NAcCAP and NPrCAP were synthesized with our expectation that the increased lipophilicity of the drug will improve the potency of drug's toxicity. They were good substrates of tyrosinase but showed slower reactions with tyrosinase-mediated oxidation. They were selectively incorporated into melanoma cells, causing irreversible DNA synthesis inhibition. Their *in vivo ip* administration also resulted in the selective accumulation of these drugs in melanoma nodules of skin and lung and their growth inhibition. NPrCAP can also be utilized for a novel cytotoxic, drug delivery system for developing hyperthermic melanoma immunochemotherapy by combining with magnetite liposome nano-particles.

IMMUNOTHERAPY FOR MELANOMA USING HYPERTHERMIA WITH MAGNETIC NANOPARTICLES AND DENDRITIC CELLS

K. Tanaka, A. Ito, T. Kobayashi, T. Kawamura, S. Shimada, K. Matsumoto, T. Saida, H. Honda,
Department of Biotechnology, School of Engineering, Nagoya University, Nagoya, Aichi, Japan, 464-8603

Dendritic cells (DCs) are potent antigen-presenting cells that play a pivotal role in regulating immune responses in cancer and have recently been shown to be activated by heat shock proteins (HSPs). It has been shown that HSPs play an important role in carrying antigens to APCs and in the maturation of DCs by acting as a danger signal. Our hyperthermia system using magnetite cationic liposomes (MCLs) induced necrotic cell death that was correlated with HSP70 release. In the present study, we investigated the therapeutic effects of DC therapy combined with MCL-induced hyperthermia on melanoma. In an *in vitro* study, when immature DCs were pulsed with B16 cells heated at 43°C for 30 min, MHC class I/II, costimulatory molecules CD80/CD86, and chemokine receptor CCR7 in the DCs were up-regulated, thus resulting in DC maturation. C57BL/6 mice bearing a melanoma nodule were subjected to combination therapy using hyperthermia and DC immunotherapy *in vivo* by means of tumor-specific hyperthermia using MCLs and directly injected immature DCs. Mice were divided into four groups: group I (control), group II (hyperthermia), group III (DC therapy), group IV (hyperthermia + DC therapy). Complete regression of tumors was observed in 60% of mice in group IV, while no tumor regression was seen among mice in the other groups. Increased CTL and NK cell activity was observed on *in vitro* cytotoxicity assay using splenocytes in the cured mice treated with combination therapy, and the cured mice rejected a second challenge of B16 melanoma cells. This study has important implications for the application of MCL-induced hyperthermia plus DC therapy in patients with advanced malignancies as a novel cancer therapy.

Kouji Tanaka, h041306d@mbox.nagoya-u.ac.jp,

Pharmaceutical Biotechnology