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メラノジェネシス標的ナノ微粒子・チロシン（フェノール）誘導体による メラノーマ化学・温熱免疫療法の開発

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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（ＤＤＳ）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ナーゼの基質であるチロシン（フェノール）誘導体の持つメラノーマへのＤＤＳと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メラノジェネシスを標的とする新しい化学・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。臨床試験開始の基盤となる動物実験の結果について報告する。

文字数　６０４字

Development of melanogenesis-targeted drug delivery and chemo-thermo-immuno (CTI) therapy for malignant melanoma using N-propionyl derivative of cysteaminyl phenol-magnetite nano-particles(NPrCAP/M).
(20 ワード以内制限で 19 ワード)

NPrCAP/M によるメラノーマ標的 DDS と化学温熱免疫療法.
(全角換算 60 文字以内制限で 24.5 文字)

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In vivo studies we utilized NPrCAP as a selective drug delivery system (DDS) and anti-melanoma agent.
Daily administration of NPrCAP/M for three times without alternating magnetic field(AMF) caused a statistically significant inhibition of growth of melanoma cells (B16F1) grown *s.c.* in C57BL mice compared to control non-treatment groups. 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. Importantly when melanoma-bearing mice on the flank were treated initially by either NPrCAP/M with or without AMF and then received second inoculation of melanoma cells at the opposite site of flank, they showed a marked growth inhibition or almost complete rejection of secondly inoculated, melanoma cells, revealing histologically the total necrosis. Thus our NPrCAP/M provided a firm basis for developing a novel, melanogenesis-targeted DDS and CTI therapy for malignant melanoma. (全角換算 500 文字以内制限で 496 文字)

Characterization of melanin in human iridal and choroidal melanocytes from eyes of various colors.

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It has been reported that the differences in iris color are mainly determined by variable melanin content in iridal melanocytes. However, whether the differences of iris coloration may also be related to the quality of the pigments present in iris tissues has notever been in detailed yet studied in detail although there is only one paper by Prota et al. Herein we present the eumelanin (EM) and pheomelanin (PM) contents in growing iridal and choroidal melanocytes from eyes of various colors (blue, green, heterochromous, yellow-brown, brown, dark brown) which were determined and quantified by chemical microanalytical procedures based on chemical degradation. The amount of EM, PM and total melanin obtained with a spectrophotometric method of choroidal melanocytes was slightly less than that of the iridal melanocytes. There is a good correlation ($r^2 = 0.554$) between total melanin obtained with a spectrophotometric method and chemical degradation methods. As there was not siginificant difference between iridal and choroidal melanocytes, we combined the data from the iridal and choroidal melanocytes as uveal melanocytes. The significant differences of total melanin were not observed in iridal and choroidal melanocytes (this may not be true, and what is TM? How about the following?) In iridal and choroidal melanocytes, EM contents were significantly higher in dark brown and brown color eyes than those in the others, On the other hand, PM contents did not show significant differences among six colors. Thus, Dark brown and brown colorr irides and choroidsiris were mostly eumelanic, while blue color irid and yellow-brown color iris choroids were pheomelanic, with green and heterochromous color eyes being intermediate. By the contrast (this is not adequate), green, and heterochromous color irids and choroids were mixed type of melanin. There

is a good correlation (r^2 = 0.554) between total melanin from spectrophotometric method and chemical degradation methods. After senescence, cultured iridal and choroidal melanocytes exhibited a marked increase in total melanin contents, most of the variation beingwas associated with the eumelanin contents. On the other hand, a significant shift to pheomelanic pigmentation was also confirmed in green and heterochromous choroidal melanocytes. (Is this correct? At least this is not consistent with the preceding sentence.) Interestingly the uveal melanoma cultured with DMEM medium, which is a cystine-rich medium, produced a large amount of pheomelanin pigment compared to F12 culture medium. (Shall we present this data? This may not be relevant to the present study.) These results suggestindicateded that that 1) Uveal melanocytes in vitro still keep their melanogenic capacity as in vivo. 2) The color of iris is mainly determined by the amount of eumelanin. 3) Tthe differences in stroma iridal and choroidal pigmentation are due not only to the quantity, as well as tobut also the typequality of the melanin presentpigment and correlate well with the visual pigmentation of iris. (words: 26549).

プログラム抄録

(和文、英文のいずれも可。演題募集要項の見本に従い、下記に上書き入力してください)

ヒトの虹彩および脈絡膜中のメラニンの定量

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６色のヒトの虹彩のヒトから単離したぶどう膜（虹彩および脈絡膜）の培養メラノサイト中のメラニン含量を測定し、虹彩の色調とメラニン量およびメラニンの型について関連があるかどうか調べた。同色の虹彩と脈絡膜の培養メラノサイト中のメラニン含量の間には有意な差はなかったので、両者の値を合算したものを使用した。ぶどう膜メラノサイト中のユーメラニン（EM）量はblue、yellow brown、green、heterochromous、brown、dark brown色になるにつれて増加した。この順序は肉眼での目で見た虹彩の色調とよく一致した。一方、フェオメラニン（PM）量は６色の間で有意な差はなかった。EM/PMの比からはEM含量と同じ増加の傾向を示し、その比はdark brownではEM量が多く、は9.3、blueではPM量が多いことがわかった。0.74であった。greenとheterochromousはその中間であった。この結果、として虹彩の色の濃さは主にEMの量によって決定され、そ虹彩の色が濃くなればなるほどEM量が多くなった。かくして、虹彩の色調の多様性はメラニン量のみならず、メラニンの型によって支配？決定されることが示唆された。

Complete Regression of Hereditary Melanoma in a Mouse Model by Repeated Hyperthermia Using Magnetite Cationic Liposomes

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Key words; magnetite, hyperthermia, liposome, melanoma, metallothionein-I/ret transgenic mouse

Magnetite cationic liposomes (MCLs) have a positive surface charge, and have been used as heating mediators for intracellular hyperthermia because they generate heat in an alternating magnetic field (AMF) due to hysteresis loss. In our previous paper, hyperthermia using MCLs was applied to animals having several types of tumor, and a strong antitumor effect was observed in those animal models using transplantable tumor cell lines. In the present study, our protocol was applied to a hereditary melanoma model; primary skin melanoma developing in a metallothionein-I (MT)/*ret* transgenic mouse line. MCLs were injected into a melanoma nodule (size, 5-7 mm) in MT/*ret* transgenic mice, which were subjected to AMF for 30 min. The temperature at the surface of the tumor reached 45°C and was maintained by adjusting the magnetic field intensity. Hyperthermia treatment was repeated three times at 24 h intervals (repeated hyperthermia; RH), and RH was carried out until complete tumor regression was observed. Complete tumor regression was achieved in all mice treated once, twice or three times with RH. Furthermore, tumors successfully treated by RH did not undergo regrowth for 120 d post-treatment, and significant elongation of survival was observed. These results suggest that MCL-mediated hyperthermia is a potent approach to treat malignant melanoma.

4-S-CAP 包埋型 MCL による悪性黒色腫に対する温熱化学療法

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悪性黒色腫はメラノーマとも呼ばれ、メラニン色素を産生する皮膚の細胞であるメラノサイトや母斑細胞が悪性化したものと考えられている。悪性黒色腫は一般的に進行が早く悪性度が高い癌とされており、外科的手術による早期治療に匹敵する有効な治療法は未だ確率されていない。我々は、これまでがんの病巣部に酸化鉄微粒子であるマグネタイトを注入し、交番磁場を照射することでマグネタイトを発熱させ 42.5℃以上の熱を加えることでがん細胞を死滅させる、磁性微粒子を発熱体とした温熱療法の研究に取り組んできた。本方法では、腫瘍内部に磁性微粒子を集積させることで他の部分には影響を与えないまま腫瘍を特異的に加温できるという特徴を持つ。また、札幌医科大学の神保孝一教授らは、メラノーマのみを特異的に治療できる化合物として、チロシンの類似体である cysteinylphenol にアミノ基を付加した 4-S-cysteaminylphenol（以下 4-S-CAP）を合成した。この 4-S-CAP はメラノーマに特異的に取り込まれ、DNA 合成系の酵素を阻害することで細胞増殖を抑制する作用を持つ。本研究では、この 4-S-CAP と MCL を組み合わせた素材である 4-S-CAP 包埋型 MCL を作製し、悪性黒色腫に対する細胞特異性と殺傷効果を高め、MCL による局所的な温熱治療の向上を目指した。

Development of Heat-chemotherapy against melanoma using 4-S-CAP enveloped MCL

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Efficient cross-presentation by heat shock protein (HSP)-antigen complex-loaded dendritic cells.

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Recent evidences have been indicating that Heat shock proteins (HSPs) play an important role as a "danger signal" in the extracellular milieu on behalf of immune surveillance. Above all, Hsp70 and Hsp90 elicit intriguing efficient CTL responses by so called "cross-presentation" with yet entirely unknown mechanism. Here, we discuss that the immunologic roles of HSPs, particularly Hsp90, in the MHC class I-restricted cross-presentation by bone marrow-derived dendritic cells (DCs). We show that Hsp90-peptide complex enter the endocytic pathway via putative Hsp90 receptor and associated peptide might be transferred onto endosomal MHC class I molecules. Moreover, we show that immunization with Hsp90-peptide complex efficiently elicits CTL responses and antitumor effect. Interestingly, this presentation is TAP-independent, but rather follows endocytic pathway. Meanwhile, when Hsp90-whole protein (OVA) antigen complex were pulsed to DCs, this protein antigen could enter at least in part via TAP-dependent pathway to the ER, and finally was presented to MHC class I molecules. However, OVA alone without Hsp90 could not enter into this pathway, but rather into MHC class II pathway.

Our data provide novel insights into the immunologic role of Hsp90 in cross-presentation of antigens, efficient induction of MHC class I-restricted CTL responses, and application to peptide/protein antigen-based immunotherapy of cancers.