2）異物発がんの経験の有無及び経験のある場合にはその内容等

上記両施設とも 異物発がんに関する経験はなかった。

3）マイクロチップの使用の有無、並びにマイクロチップによる発がんの経験の有無及び経験等

上記両施設とも、手術を行なう動物へのマイクロチップの使用はないとの回答であった。

昨年度の本邦のアンケート調査では、非 GLP 下で実施される薬理試験及び薬物動態試験の結果により収集された SPF 下でのげっ歯類の手術環境については、以下のとおりであった。

①手術部位の皮膚の前処置（消毒）に SPF 環境及びコンベンショナル環境ともに 70%エタノールが 56～57%程度用いられていた。なお、ヒトの場合で殆どがポピドンヨード又はクロルヘキシジン（ヒビテン）が用いられている。その理由はその効果がアルコールより高いこととされており、アルコール単独の消毒では手術部位の皮膚の前処置が不十分である可能性が考えられる。

②切開部位以外の部位からの感染をさけるために切開部位以外の部位を覆布で覆うことは殆ど行なわれていない。

③手術機器、特に動物に直接触れるピンセット、ハサミやメス等についてはヒトでは滅菌されたものが使用されているが、マウスやラット等のげっ歯類では SPF 環境の場合においても滅菌（乾熱滅菌、高圧蒸気滅菌及びガス滅菌）は 72%、消毒（アルコール、ヒビテン等）は 28%であった。また、これらの手術機器は同一動物での再使用のみならず、同一実験中で他の動物にも再使用されていることから、これらの手術機器による感染等の可能性が考えられる。

④術後の創傷の管理としてげっ歯類では通常は抗菌剤の投与はせず、また、切開創を被覆剤で保護しない。つまり、傷口からの感染の防御が不十分な可能性が高い。

⑤動物自身が舐める又は別の個体に舐められる等して、傷口からの細菌（口内細菌や皮膚常在菌等）感染が起きる可能性について検討するために必要な長期飼育の動物に関する情報が不足しているが、本アンケート調査の回答によれば多くの場合は 1 動物が 1 ケージに飼育されていたため、他の動物により傷をなめられる可能性は低いと考えられる。ただし、その場合でもエリザベスカラーをしていな場合が殆どであり、手術後の保定器具により身動きできない場合及び創傷の位置が動物自身でなめることができない場合を除き、動物自身がなめることによる傷口からの感染の可能性は否定できない。

一方、本年度の施設については、

infusion 試験のためのカニューレを留置する手術を見学した結果、昨年度の本邦試験施設のアンケート調査結果とは、以下の点が異なっていた。なお、これらの事項は昨年度の本邦におけるアンケート調査の結果から、GLP 施設等における SPF マウスやラットの手術に関して、ヒトの術後感染の制御の観点から注意が必要と考えられた点であった。

① 手術前の動物の手術部位の消毒（清拭）に際して、1 剤ではなく 2 又は 3 剤を使用していた。

＊Charles River 社：グルコン酸クロルヘキシジンを使用後、イソジンを使用

＊ITR 社　：グルコン酸クロルヘキシジンを使用後、アルコールを使用し、さらにイソジンを使用

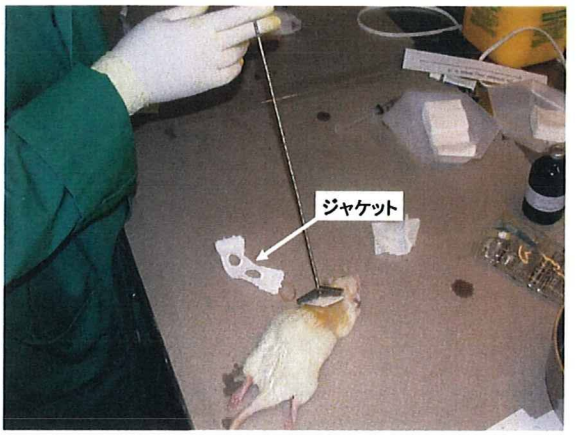
用いられた消毒薬は、それぞれ特徴が異なることから、これらを組み合わせて用いることにより、高い消毒効果が期待できると考えられる。

② 手術中に使用した滅菌手術機器（動物に直接触れるもの）の再使用に関して、他の動物への使用はなく、同一動物に使用する際にも消毒が必ず行なわれていた。

③ 切開部位以外の部位からの感染等を防ぐため切開部位以外を覆布で覆っていた。

④ 術中又は術後感染管理のために抗菌剤が使用されていた。また、術後はすべて 1 動物が 1 ケージに飼育されていた。

⑤ ITR 社においては、手術後に滅菌済みのジャケットを動物に装着させることにより傷口からの感染を防いでいた。



以上の結果から、調査対象となった施設においては、手術室や手術時の無菌性並びに術後の創傷管理等がヒトの手術とほぼ同等のレベルであると考えられた。また、調査対象施設においては、手術室の出入り等についてもヒトと同等に管理されていた。このため、当該施設では細菌感染による異物発がんの可能性は低いと考えられる。

<u>文献調査について</u>

本年度は、昨年度に引き続きげっ歯類の異物発がん、感染／炎症による発がんに関する情報等を収集したが、特に注目すべき情報はなかった。

実験動物の倫理的取り扱いに関する規制（AAALAC；Association for Assessment and Accreditation of

Laboratory Animal Care International、CCAC ; Canadian Council on Animal Care 等による施設認証）においても手術等に関する規定があり、欧米では AAALAC 等の認証を受けないと実験動物を使用することができない状況である。このため、当該情報についても入手した。回復が必要な手術の際の術野の無菌性（皮膚の消毒、手術時の無菌性等）並びに術後感染制御等に関する記載は、以下のとおりであった。

① 無菌的に手術を行なうために必要な設備

a）手術に供する動物を準備するエリア

b）ヒトが手術の準備をするエリア

c）手術室

d）術後の動物の回復用のエリア

e）手術をサポートするエリア（手術に関する用具が機器の供給、洗浄、滅菌等）

② 手術室の環境等

a）通常の施設から離れていること。

b）ヒトの導線が交わらないこと。

c）設備や床、内壁等が容易に洗浄又は清浄にできること。

d）周囲の施設に比べて陽圧であること。

e）空気を循環させないこと。

f）流入する空気は、適切はフィルターを用いる等して、清浄なものとすること。

③ 手術前の準備

a）手術担当者は、無菌的な手術の術技を含めて手術に関して十分訓練されていること。

b）適切なプロトコールを作成し、術者とそれ以外のスタッフの連携がスムースに行くようにすること。

c）術前の動物処置、術技（麻酔を含む）、術後のケアに関して、獣医師に相談すること。獣医師は、それらが適切に行なわれていることを確認すること。

d）健康な正常動物のみを手術に供すること（健康な SPF 動物を使用すること）。

e）動物の順化を適切に行なうことにより、動物のストレスを減らすこと。

④ 手術

a）無菌的に実施すること。

b）手術に用いる機器や器具（埋設材料。カニューレ、テレメトリー機器、埋設医療機器等）は、すべて必ず滅菌すること。

c）術者は、スクラブを用いて手を洗い、手術用キャップ、マスクを着用すること。さらに、滅菌済みの手術着及び手袋を着用すること。

d）手術は、できる限りクリーンな環境で、滅菌された道具と滅菌手袋を用いて、無菌的に行なうこと。

e) 感染を最小限に抑える努力をすること。

f) ラットは他のげっ歯類に比べて術後感染が低いと考えられているが、十分に滅菌されていない埋設材料（カニューレ等）を用いることや無菌的に手術をしないこと等は許容されない。

g) 手術に用いるピンセット、メス、ハサミ等は複数滅菌し、再使用を避けること。再使用する場合には、消毒液に浸しておくこと。

本年度実地に調査した２施設はいずれも AAALAC 及び CCAC の認証を受けており、上記の事項は遵守されていた。

参考までに、ITR 社における手術室や術者等に関する写真を示す。

ヒトの手術の場合と同様に、術者は滅菌手袋で手術道具以外に触れることができないため、術着の着用を補助者が補助している。

海外の GLP 適合施設に対するアンケート調査票の作成について

さらに埋設医療機器等に関する手術時の術野の無菌性（皮膚の消毒、手術時の無菌性等）並びに術後感染制御等に関する情報等を調査するために、来年度は海外の GLP 適合施設に対するアンケート調査を予定している。本年度は、昨年度実施した日本の GLP 適合施設に対するアンケート調査票を基にアンケート調査票（案）を作成した（添付資料１参照）。なお、今般の調査結果を踏まえた改定等（調査対象試験、調査項目等）は来年度実施する。

まとめ

実験動物の飼育環境、手術時の術野の無菌性（皮膚の消毒、手術時の無菌性等）並びに術後感染制御等に関する情報を得るために、昨年度は本邦の GLP 適合施設等を有する法人へのアンケート調査を行なったが、医療機器の埋植試験等に関する情報を十分得ることができなかった。このため、本年度は、GLP に適合した海外（カナダ）の安全性試験受託施設での手術を伴う試験（infusion 試験におけるカニューレの留置のための手術）に関する実地調査及び情報収集を行った。

この結果、①手術部位の消毒に複数の消毒薬を用いること、②動物に直接触れる手術機器の再使用は同一動物にしか行なっていないこと及び再使用する場合には機器を消毒すること、③切開部位以外を覆布で覆っていること、並びに④術中及び術後感染制御のために抗菌剤を一定

期間使用していること、さらに⑤調査した施設の１つは傷口からの感染を防ぐため滅菌したジャケットを術後の動物に着用させること等が、昨年度のアンケート調査の結果とは異なっていた。これら①～⑤の事項は、昨年度のアンケート調査の結果からで、手術環境において改善が必要と考えられた事項であった。本調査の結果、調査対象となった施設における実験動物の手術時の術野の無菌性（皮膚の消毒、手術時の無菌性等）、並びに術後感染制御等はヒトとほぼ同等であることが明らかになった。

来年度は、本年度作成したアンケート調査票（案）を今般の調査に基づいて改定し、当該調査票を用いて、さらに多くの海外の GLP 適合施設における埋設医療機器等に関する手術時の術野の無菌性（皮膚の消毒、手術時の無菌性等）、並びに術後感染制御等に関する情報等を調査する予定である。

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2) 大内希、大室弘美、吉田ルシア幸子、懸川友人

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第83回日本薬理学会年会（一般演題；2010年3月16日、大阪)

G. 知的財産所有権の出願・登録状況（予定も含む)

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

添付資料

2010

To Whom It May Concern:

A questionnaire survey on environmental and pre- or post-surgical issues about animal experimentation

Hiromi Ohmuro
Department of Pharmaceutical Information, School of Pharmacy, Musashino University

Dear Madam or Sir,

In a research program entitled "Research into Biological Safety Evaluation of Materials for Self-Contained Medical Devices – Reevaluation Centering on Carcinogenicity and International Harmonization" – funded by the Ministry of Health, Labour and Welfare (Representative Researcher: Seiji Sekita, National Institute of Health Sciences) (2008 - 2010), we have been investigating various aspects of the possibility that the "bacterial coexistence environment" is a factor that leads to foreign material-induced carcinogenesis that is observed often in rodents.

I am one of the co-researchers participating in the above Research Program, and I plan to perform this survey to obtain information on the rearing environments of experimental animals, the sterility of surgical field (skin disinfection, intraoperative sterility, etc.), prevention of postoperative infection and related matters.

The contents of this survey are mainly items related to the surgical environment, as described in the "Guideline for the Prevention of Surgical Site Infection (CDC; Center for Disease Control and Prevention)" intended for comparison with surgical operations performed in humans. Furthermore, some questions are related to your experience of foreign material-induced carcinogenesis and microchip embedding.

I must apologize for taking up your precious time, but I would be most grateful if I could obtain answers from each section (unit) engaged in surgical operations that involve the use of animals (rodents and non-rodents) at your institution.

Thank you in advance for your cooperation.

Sincerely yours

Request on filling in the questionnaire

Please follow the instructions below before completing the questionnaire.

1. In the case your institution having multiple units or sections dealing with experimental animals (e.g., toxicology section, pharmacology section, pharmacokinetics section), please have each section representative answer the questionnaire. In this case, I ask you the favor of making necessary copies for each section.
2. Please circle the applicable item or write the answer in the pertinent column.
3. Please provide just one answer unless otherwise specified.
4. Your answers will never be used for any purposes other than this survey, but the results of this survey may be presented in academic meetings or publications for academic purposes, protecting the confidentiality of company information.
5. Please submit the answers by MM DD.

[Contact information for this questionnaire survey]

Contact information for inquiries about the purpose and contents of this questionnaire:

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Question 1 About the studies/tests/experiments performed at your section

in the following, if the term "your section" is used, please read this term as "the department, laboratory, unit, etc. where you are working".

If multiple answers are applicable in A. and B. below, please use multiple, separate questionnaires. If there is insufficient space, please take a few minutes to copy the form in order to provide a complete answer.

A. What type of study is performed in your section?

1. Toxicology (drugs) 2. Toxicology (medical devices) 3. Pharmacology
4. Pharmacokinetics 5. Other ( )

Note: Please select one study type from the above. If multiple study types are applicable, please take a few minutes to copy the form, in order to provide multiple separate answers.

B. What animal species is used in the study mentioned in A. above?

1. Mouse 2. Rat 3. Rabbit 4. Dog 5. Cat 6. Other ( )

Note : (i) If you use multiple animal species, please provide multiple answers. In such cases, please answer the subsequent questions, which apply mainly to rodents.

(ii) If the rodent species you use include mice and rats, please provide answers about mice, and please describe just the differences between mice and rats in the margin of the part concerned, in a manner that is easy to understand.

(iii) If you use a non-rodent species in addition to a rodent species and the subsequent answers are the same as the answers for mice, please describe just the differences in the margin of the part concerned, in a clear fashion.

(iv) If you use a non-rodent species in addition to a rodent species and the subsequent answers are different from the answers for the rodent species, please take a few minutes to copy the form in order to provide multiple separate answers.

(v) If you use multiple non-rodent species, please provide answers in a similar fashion to (ii), (iii) or (iv) above.

C. Which of the following is applicable to the rearing environment for the animal

species mentioned in B. above?

1. SPF 2. Conventional

D. If the environment in which the animals are reared is SPF, the items shown in the following table are expected to be monitored.

Please refer to the table below for your information.

Target	Purpose of monitoring	Items to be monitored
Facility	To check whether the barrier is functioning effectively	Inter-room differential pressure, air flow direction, high-pressure steam sterilization, HEPA filter, etc.
Environment	To confirm that the animals are kept under constant physiological conditions and are not stressed	Temperature, humidity, lighting hours, noise, illuminance, etc.
	To check the cleanliness of the facility	A decline in the bacterial count, ventilation frequency, dust count, ammonia concentration, etc.
	To check whether any chemical substance that might influence the study is ingested	Contaminants in the feedstuff, drinking water and cage litter
Animal	Confirmation of SPF status	Specific pathogens

* Please answer the following items related to monitoring of the rearing environment.

1. Type of HEPA filter_____
2. Decline in the bacterial count _____
3. Timing to measure a decline in the bacterial count

   _____
4. Method used to sterilize the drinking water_____
5. Special pathogens to which particular attention is paid within your section, if any

   _____
6. Please specify pathogens which routinely monitored.

   _____

   _____

   _____

Question 2 About surgical environment

A. Do you remove the hair of animals before surgical operations?

1. Yes

1-1 When do you remove the hair?

(i) Immediately before the operation (ii) At least 1 hour before operation

(iii) Other ( )

1-2 How do you remove the hair?

(i) Shaving with a razor (ii) Depilation agent (name:)

(iii) Other ( )

2. No

(Reason:)

B. Pretreatment of the skin at the surgical site

1. Which of the following disinfectants do you use? How many minutes do you wait for after wiping the skin, until you incise the skin? (Multiple answers are acceptable)

(i) 70 v/v% ethanol (left to stand for about ___ minutes)

(ii) Povidone-iodine (concentration or brand name_____, left to stand for about ___ minutes)

(iii) Chlorhexidine (concentration or brand name_____, left to stand for about ___ minutes)

(iv) Other

(Ingredient name: _____, concentration or brand name_____, left to stand for about ___ minutes)

2. How extensive is the area disinfected?

(i) Concentric disinfection from the planned incision site to a sufficient area in consideration of possible expansion of the incision area

(ii) An area a little bit wider than the planned incision area

(iii) Other ( )

C. Disinfection of hands, fingers, etc. for surgical personnel

1. Please encircle the applicable one(s).

(i) Are hands washed with disinfectant?

If "yes", please name the disinfectant: _____

(ii) Is there application of disinfectant up to the level of the elbows?

a. Yes b. No

(iii) Do you use scrub?

If "yes", please circle the site to be scrubbed (multiple answers are acceptable)

a. Fingers b. Hands c. Elbows d. Other ( )

(iv) Other (methods other than the above):)

2. Use of surgical attire, masks and gloves

(i) Surgical attire

a. Worn b. Not worn

(ii) Masks

a. Worn b. Not worn

(iii) Sterile gloves

a. Worn b. Not worn

(iv) Other ( )

D. The Operating room environment

1. Is there a room dedicated to surgical operations?

(i) Yes (ii) No (iii) Other ( )

2. How many times is the air exchanged in the operating room per hour?

__ times

3. Are HEPA filters used in the operating room?

(i) Yes

Type of HEPA filter ( )

(ii) No

(iii) Other ( )

4. How do you disinfect the operating room? (Multiple answers acceptable)

(i) Periodic disinfection

Name of disinfectant used ( )

Method of disinfection: a. Wiping b. Spray c. Other ( )

(ii) Disinfection only in the case of contamination

Name of disinfectant used ( )

Method of disinfection: a. Wiping b. Spray c. Other ( )

(iii) Disinfection between surgical operations

Name of disinfectant used ( )

Method of disinfection: a. Wiping b. Spray c. Other ( )

(iv) Other ( )

Name of disinfectant used ( )

Method of disinfection: a. Wiping b. Spray c. Other ( )

Question 3 About intraoperative and postoperative prevention of infection

A. How do you sterilize or disinfect the surgical instruments (mainly those in direct contact with animals)? Multiple answers are acceptable.

(i) Dry-heat sterilization (Targets: , etc.)

Sterilizing conditions: ___°C, ___ minutes

Method used to confirm sterilization ( )

(ii) High-pressure steam sterilization (Targets: , etc.)

Sterilizing conditions: ___°C, ___ minutes

Method used to confirm sterilization ( )

(iii) Other (Targets: , etc.)

Sterilizing conditions: ___°C, ___ minutes

Method used to confirm sterilization ( )

B. Do you reuse surgical scalpels, scissors, tweezers, etc. that have been used previously, during the same operation?

1. No
2. Yes (to be reused in the same animal)
   (i) To be reused without disinfection
   (ii) To be reused with disinfection_ (iii)
   (iii) How do you disinfect for reuse?
   a. Disinfection with alcohol b. Dipping in alcohol followed by heating with a burner
   c. Other ( )
3. Yes (to be reused, even in other animals during the same series of experiments)
   (i) To be reused without disinfection
   (ii) To be reused with disinfection_ (iii)
   (iii) How do you disinfect for reuse?
   a. Disinfection with alcohol b. Dipping in alcohol followed by heating with a burner
   c. Other ( )
4. Other ( )

C. How do you sterilize or disinfect the material to be imbedded?

Please answer if you have relevant experience. (Multiple answers acceptable)

(i) Dry-heat sterilization (Targets: , etc.)
Sterilizing conditions: ___°C, ___ minutes
Method used to confirm sterilization ( )
(ii) High-pressure steam sterilization (Targets: , etc.)
Sterilizing conditions: ___°C, ___ minutes
Method used to confirm sterilization ( )
(iii) Other (Targets: , etc.)
Sterilizing conditions: ___°C, ___ minutes
Method used to confirm sterilization ( )

D. In order to prevent infection of the incised part during operation, do you cover other parts with a cloth or something similar?

(i) Yes (ii) No (iii) Other ( )

E. How do you manage the surgical wound after operation?

1. How do you close the surgical wound?
   (i) Not closed
   (Reason:)
   (ii) Closed with a surgical adhesive agent (brand name, etc.:)
   (iii) Closed with clips, etc. (brand name, etc.:)
   (iv) Other ( )
2. Do you protect the incised wound with a sterile cover?
   (i) No (reason:)
   (ii) No protection where the wound has been closed with a surgical adhesive agent
   (iii) No protection where the wound has been disinfected
   (Brand name of disinfectant:)
   (iv) Protected with a cover
   (Name of cover, etc.:)
   (Covering method:)
   (v) Other ( )

F. Do you use any antibiotic agent(s) to prevent intraoperative or postoperative infection?

(i) No
(ii) Yes _ (iii)
(iii) In which situations [do you use an antibiotic]? _ (iv)

( )

(iv) In the case you use an antibiotic agent, how long do you use it for?

( )

(v) Other comments ( )

G. How do you rear the animals after surgical operations?

([We wish to determine][The information required is related to] whether the animal itself can lick the wound or whether other animals can lick the wound)

(i) After operation, multiple animals are accommodated in the same cage.

(ii) After operation, a single animal is accommodated in each cage.

(iii) After operation, a single animal is accommodated in each cage after putting on an Elisabeth Collar so that the animal cannot lick the wound.

(iv) After operation, a single animal is accommodated in each cage until the wound closes, and thereafter multiple animals are accommodated in the same cage.

(v) After operation, a single animal is accommodated in each cage after putting on an Elisabeth Collar until the wound closes, and thereafter multiple animals are accommodated in the same cage.

(vi) Other ( )

Question 4 About foreign material-induced carcinogenesis

A. Please answer the following questions if you have any relevant experience.

1. Please describe the animal species (and strain) that developed foreign material-induced carcinogenesis, type of foreign material, elapsed time until onset, type of cancer, etc. to the best of your knowledge.

_____

_____

_____

_____

2. If the surgical environment of the animal that developed foreign material-induced carcinogenesis was different from the answers to Question 2 (A, B and C) and Question 3 (A, B and C), please describe the differences below.

_____

_____

_____

_____

_____

_____

_____

A. Are microchips used in your section?

a. No b. Yes

B. Please describe the intended use of microchips and the number of animals imbedded with microchips, to the best of your knowledge.

Species and strain of animals imbedded with microchips	Intended use (e.g., individual identification)	Number (e.g., ____ animals/year, ___ animals in ___ years)

C. Please answer the following questions about the method used to disinfect the instruments and skin on embedding microchips.

1. Do you disinfect the skin?
   (i) No (ii) Yes _ 2.
2. Which of the following disinfectants do you use? How many minutes do you wait for, after wiping the skin, until incising the skin? (Multiple answers acceptable)
   (i) 70 % w/v ethanol (left to stand for about ___ minutes)
   (ii) Povidone-iodine (concentration or brand name_____, left to stand for about ___ minutes)
   (iii) Chlorhexidine (concentration or brand name_____, left to stand for about ___ minutes)
   (iv) Other
   (Ingredient name: _____, concentration or brand name_____, left to stand for about ___ minutes)
3. How extensive is the area disinfected?
   (i) Concentric disinfection from the planned incision site to a sufficient area in

consideration of possible expansion of the incision area

(ii) An area a little bit wider than the planned incision area

(iii) Other ( )

D. Please complete the following table to the best of your knowledge, if you have any experience related to microchip-induced carcinogenesis.

Species and number of animals, Incidence rate of microchip-induced carcinogenesis	Type of cancer	Elapsed time until carcinogenesis and age in weeks at the final onset of cancer	Rearing environment (SPF or conventional)

The end

Thank you for your cooperation.

別添　5

III. 研究成果の刊行に関する一覧表

該当無し。

IV.　研究成果の刊行物・別冊

該当無し。

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8-Nitroguanine as a potential biomarker for progression of malignant fibrous histiocytoma, a model of inflammation-related cancer

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Abstract. Chronic inflammation is a critical component of carcinogenesis and tumor progression. Reactive nitrogen and oxygen species generated by inflammatory cells form mutagenic DNA lesions, such as 8-nitroguanine, which may play an integral role in inflammation-related carcinogenesis. Hypoxia-inducible factor (HIF)-1α has been established as a prognostic biomarker in various tumors, including malignant fibrous histiocytoma (MFH). The aim of this study was to evaluate the impact of 8-nitroguanine formation and HIF-1α expression on the prognosis of patients with inflammation-related cancer. Immunohistochemical analyses were employed to examine the distribution of 8-nitroguanine and HIF-1α, using clinical specimens from 36 patients with MFH as a model of inflammation-related cancer. 8-Nitroguanine formation was predominately observed in the nuclei of tumor cells and inflammatory cells in tumor tissues, while HIF-1α was expressed in the cytoplasm and nuclei of tumor cells. Little or no immunoreactivity of 8-nitroguanine and HIF-1α was observed in adjacent non-tumor tissues. Significantly higher levels of both 8-nitroguanine and HIF-1α were observed in the tissue specimens of deceased patients than in those of living subjects. Survival curves analyzed by the Kaplan-Meier method differed significantly between the high- and low-staining groups of 8-nitroguanine (p=0.00003) as well as HIF-1α (p=0.01104). These results suggest a significant role of the pathway of iNOS-dependent 8-nitroguanine formation via HIF-1α and NF-κB on the progression of inflammation-related cancer. In conclusion, 8-nitroguanine is an excellent candidate prognostic and predictive biomarker together with HIF-1α in inflammation-related tumor progression.

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Introduction

Inflammation is a critical component of carcinogenesis and tumor progression (1). Many malignancies arise from inflammatory sites, and chronic inflammation contributes to the development of various cancers (1,2). In cases of chronic inflammation, reactive nitrogen species (RNS) and reactive oxygen species (ROS) are generated by inflammatory cells and the epithelium (3,4). RNS mediate 8-nitroguanine formation, a marker of nitrative DNA damage (5). 8-Nitroguanine has been reported to be formed in association with inflammation-related carcinogenesis (6-9), including malignant fibrous histiocytoma (MFH), as reported previously (10). MFH is the most commonly diagnosed soft-tissue sarcoma in adults (11,12) and has a poor prognosis (13,14). Several studies have shown that hypoxia-inducible factor (HIF)-1α could be a biomarker of a poor prognosis in various cancers (15-17), including soft-tissue sarcomas (18). The HIF-1α protein supports the adaptation of human cancer cells to hypoxia under tumor growth.

This study investigates 8-nitroguanine formation and HIF-1α expression in surgical specimens of MFH patients using immunohistochemical staining procedures. We evaluated the impact of 8-nitroguanine formation and HIF-1α expression on prognosis, and examined their usefulness as potential biomarkers.

Materials and methods

Tissue preparation and clinicopathological analysis. Thirty-six MFH patients who underwent an open biopsy or a surgical resection from 1989 to 2004 at the Department of Orthopaedic Surgery, Mie University Graduate School of Medicine, Japan, participated in this study, which was approved by the Ethics Committee of Mie University Graduate School of Medicine. All patients were diagnosed by well-trained pathologists, according to the Enzinger and Weiss classification (19). The

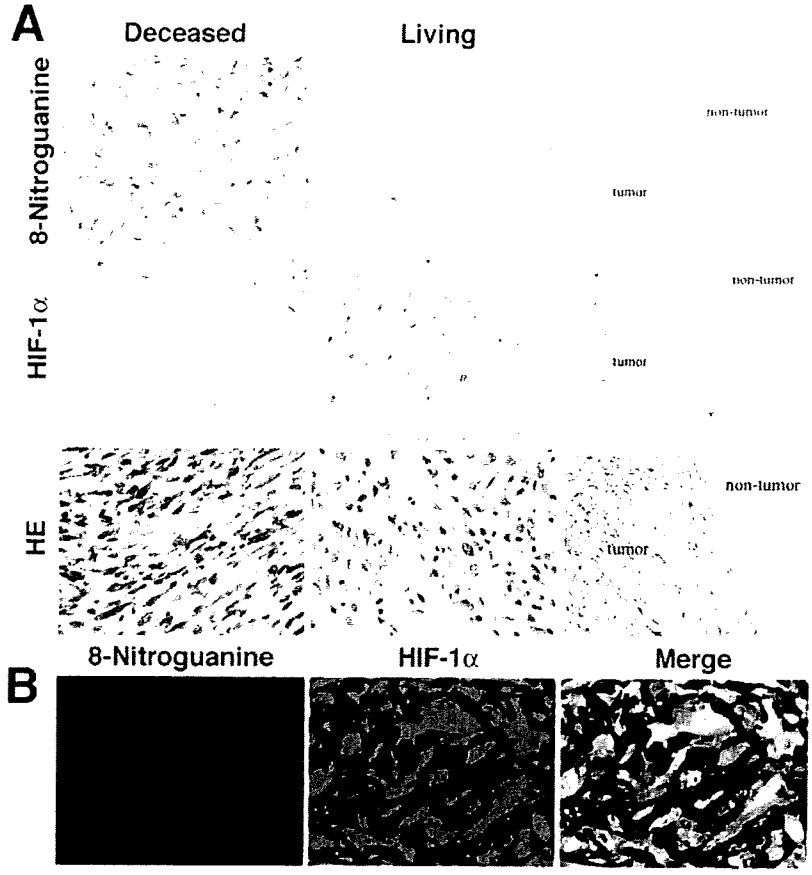

Figure 1. 8-Nitroguanine formation and HIF-1α expression in MFH patients. (A) 8-Nitroguanine formation and HIF-1α expression by immunohistochemical staining using the LSAB method and hematoxylin and eosin staining. Both are observed in the nuclei of tumor cells and inflammatory cells within MFH tissue specimens. The immunoreactivity of 8-nitroguanine and HIF-1α is found to be greater in deceased patients than in living subjects. Little or no immunoreactivity of 8-nitroguanine and HIF-1α is observed in adjacent non-tumor tissues. Magnification, x400 (left and center columns) and x100 (right column). (B) The detection of colocalization of 8-nitroguanine with HIF-1α by double immunofluorescence staining. 8-Nitroguanine and HIF-1α are colocalized in the same tumor cells. Magnification, x880.

patients comprised 20 men and 16 women ranging in age from 27 to 85 years (mean ± SD, 63.0 ± 13.0 years). Survival data were available for all patients. The duration of the follow-up ranged from 4 to 213 months (median, 63 months). The tumor samples were classified as Stage IIa (2 patients), Stage IIb (3 patients), Stage III (25 patients) and Stage IV (6 patients) at the time of the original diagnosis. The International Union Against Cancer TNM classification and the staging system by the American Joint Committee on Cancer (AJCC) were used for tumor assessment (20). One of 2 patients in Stage IIa, none of 3 patients in Stage IIb, 9 of 25 patients in Stage III, and all of 6 patients in Stage IV died. Thirty-four tumors measured 5-22 cm in diameter, while the other 2 tumors were <5 cm in diameter (mean ± SD, 11.15 ± 4.48 cm in diameter).

Immunohistochemical analysis for 8-nitroguanine and HIF-1α. Immunohistochemical staining was performed using the labeled streptavidin-biotin (LSAB) method. The sections were deparaffinized and automated immunohistochemistry was performed with a NexES IHC (Ventana Medical Systems, Inc., Tucson, AZ, USA) as previously described (10). The rabbit polyclonal anti-8-nitroguanine antibody produced by this laboratory (21) was used as the primary antibody at a concentration of 2 μg/ml. The mouse monoclonal anti-HIF-1α antibody (Calbiochem-Novabiochem, Darmstadt, Germany) was diluted at 1:500. As secondary antibodies, anti-mouse IgG and anti-rabbit IgG antibodies (Ventana Medical Systems, Inc.) were used. The Lumina Vision version 1.11 software program (Mitani Shoji Co., Fukui, Japan) for performing morphometric analyses was used to measure the staining rates of 8-nitroguanine and HIF-1α.

To examine the colocalization of 8-nitroguanine formation and HIF-1α expression, a double immunofluorescence technique was used, as described previously (10). The stained sections were examined under a fluorescence microscope (BX50F-3, Olympus Optical Co., Ltd., Japan).

Histopathological staining. A histopathological study was performed, following the standard method, using hematoxylin and eosin staining in paraffin sections .

Statistical analysis. The patients were categorized into five subgroups according to the staining rates (<7.5, 7.5-15.0, 15.0-22.5, 22.5-30.0 and >30.0%) and evaluated as described above. Then, statistical differences of the immunoreactivities between deceased and living patients were analyzed by χ^2-test. Survival between the two subgroups, high-grade (staining rates, ≥15%) and low-grade (<15%), was compared using the