欠損放置群では、軟骨欠損部は何らかの修復組織で覆われているが、陥凹しており、修復は不十分である。

担体 SZ のみでは、軟骨欠損部は周辺の性状軟骨と同じレベルまで修復組織で覆われている。しかし、修復部の表面は不整である。

担体 SZ ＋骨髄間葉系細胞群では、軟骨欠損部は周囲の正常軟骨と類似した性状を持つ組織で覆われており、表面も整である。
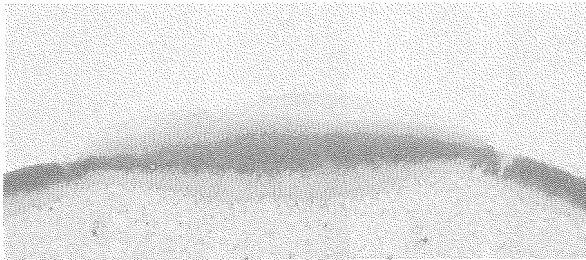
組織学的所見. トルイジンブルー染色
【担体 SZ のみ】

欠損部の深部はメタクロマジーを示す組織で埋められているが、鏡面の染色性は悪く、表層は不整である。周辺の正常軟骨部に変性が認められる、

【担体 SZ ＋骨髄細胞】

表層のメタクロマジーが悪いものの、欠損部全体にメタクロマジーが認められる。表層も整である。周辺の正常軟骨部は痛んでいない。

D．考察
ミニブタを使った関節軟骨欠損モデルでヒトに類似した手術を施行できることが明らかになった。膝関節の形状がヒトに類似し、大きさも近いので、欠損を作成し、担体の縫合もヒトと同様に施行可能である。

評価方法もヒトと同様に、Magnetic resonance imaging (MRI) 施行可能である。また組織が大きいので、様々な力学的試験の施行が可能である。これらのデータと組織学的評価を比較し、その相関を明らかにする。それにより、組織標本を採りにくいヒトでの修復を、MRI や力学的試験で類推できるようになると考えられる。

E．結論
ミニブタをヒト類似動物モデルとして使用できるかを評価した。
1．ミニブタに対してヒトと同じ軟骨欠損修復法を施行可能であった。
2．ミニブタに対してヒトと同じ評価法 (MRI、力学的試験など) の施行が可能であった。
3．担体 SZ ＋自己骨髄間葉系細胞移植が良好な修復を得られた

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G．知的財産権の出願・登録状況
　1．特許取得　　　　　　なし
　2．実用新案登録　　　　なし
　3．その他　　　　　　　なし

III　研究成果の刊行に関する一覧表

別紙4

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IV 研究成果の刊行物・別刷り

Effects of a biodegradable polymer synthesized with inorganic tin on the chondrogenesis of human articular chondrocytes

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Abstract: Recent study has shown that biodegradable polymers are attractive candidates for chondrocyte fixation and further transplantation in cartilage tissue engineering. Poly (glycolic acid) (PGA), a polymer of glycolic acid, is widely used in orthopedic applications as a biodegradable polymer. Organotin, lead, antimony, and zinc are catalysts commonly used in synthesizing PGA. Here, we investigated the biocompatibility of PGA, synthesized with and without inorganic tin as a catalyst in chondrogenesis of human articular chondrocytes in a micromass culture system. Significant enhancement of chondrocyte proliferation and expression of the collagen type II protein gene were observed in cultures treated with PGA synthesized with a tin catalyst. However, aggrecan gene expression was very similar to the control culture. Amount of collagen type II protein was also increased in the same group of cultured chondrocytes. In contrast, PGA without a catalyst caused overall inhibition of chondrogenesis. Despite several positive findings, extensive investigations are essential for the feasibility of this PGA(Sn) in future clinical practice. © 2005 Wiley Periodicals, Inc. J Biomed Mater Res 77A: 84–89, 2006

Key words: poly (glycolic acid); inorganic tin catalyst; human articular cartilage; chondrogenesis; micromass culture

INTRODUCTION

Different synthetic biodegradable polymers are currently gaining importance in the fields of biotechnology and tissue engineering. Recently, many studies have evaluated the potential of various natural bioabsorbable polymers such as collagen, [1,2] alginates, [3–5] fibrin, [6–8] and gelatin, [9] but synthetic biodegradable polymers in general offer advantages over natural materials. The primary advantages include the capacity to change the mechanical properties and degradation kinetics to suit various applications. Among the families of synthetic polymers, polyesters are used in a number of clinical applications. [10–12] Polyesters have also been used for development of tissue engineering applications, [13,14] particularly for bone tissue engineering. [15,12]

The attraction of poly (glycolic acid) (PGA), one of the aliphatic polyesters, as a biodegradable polymer in medical applications is that its degradation product, glycolic acid, is a natural metabolite. Several studies have indicated that copolymers of glycolic acid caused promotion of nerve regeneration in a rat model, [16–18] and regeneration of an 80 mm nerve gap by an artificial nerve conduit made of PGA was also reported. [19] PGA can be synthesized using different catalysts. The common catalysts used include organotin, lead, antimony, and zinc. It was reported that inorganic and organic tin compounds present in the aqueous ecosystem have toxic effects and are capable of producing behavioral abnormalities in living organisms. [20,21] Organotin compounds are known to cause neurotoxicity, [22] cytotoxicity, [23] immunotoxicity, and genotoxicity [24] in human and other mammalian cells both *in vitro* and *in vivo*. Organotin compounds were also reported to decrease *in vitro* survival, proliferation, and differentiation of normal human B cells. [25] The dose effect of inorganic tin in rats suggests that the critical organ in inorganic tin toxicity is bone, [26] and disproportionate dwarfing syndrome, which severely affects the limbs but not the trunk, was observed in rats that had been injected with certain tin compounds. [27] As far as we know, no study yet has reported the chondrogenic

effects of PGA synthesized with and without an inorganic tin catalyst. In this study, the biocompatibility of PGA with and without a tin catalyst was investigated, using human articular chondrocytes (HAC) in a micromass culture system.

MATERIALS AND METHODS

Medium and polymers used for cell culture

Chondrocyte growth medium was obtained commercially from BioWhittaker (Walkersville, MD, USA). PGA synthesized with inorganic tin [PGA(Sn)] (M_w = 1500) and without a catalyst (PGA) (M_w = 1100) were custom-made (TAKI chemicals, Kakogawa, Japan) and dissolved in dimethyl sulphoxide (DMSO) (Sigma Chemical, St. Louis, MO, USA).

Cells and culture methods

Human articular chondrocytes (HAC) of the knee joint was commercially obtained from BioWhittaker. High-density micromass cultures were started by spotting 4×10^5 cells in 20 μL of medium onto Costar 24-well tissue culture microplates (Costar type 3526, Corning). After a 2 h attachment period at 37°C in a CO_2 incubator, culture medium (1 mL/well) was added to each well. Media were supplemented with DMSO (0.8 μL/mL), PGA, and PGA(Sn) (50 μg/mL). HAC cultured with DMSO was used as the control. The cultures were continued for 4 weeks with a medium change twice a week. At least four cultures were performed for each sample.

Cell proliferation study

Cell proliferation was quantitatively estimated by crystal violet (Wako Pure Chemical Industries, Osaka, Japan) staining, as previously described.[28] After the culture period, cells were fixed with 100% methanol at room temperature, followed by application of 0.1% crystal violet in methanol. After a proper wash, cells were again incubated in methanol; 100 μL from each well was transferred to a new 96-well plate, and the absorbance was measured at a wavelength of 590 nm, using an ELISA reader (Bio-Tek Instruments, Winooski, VT). Blank values were subtracted from experimental values to eliminate background readings.

Differentiation assay

Cell differentiation assay was performed by alcian blue (Wako Pure Chemical Industries, Osaka, Japan) staining, as previously described.[29] Following crystal violet staining, the cells were washed with methanol and then 3% acetic acid.

Cultures were then stained with 1% (v/v) alcian blue in 3% acetic acid, pH 1.0. The cartilage proteoglycans were extracted with 4M guanidine hydrochloride (GH), and the bound dye was measured at wavelength of 600 nm, using an ELISA reader (Bio-Tek Instruments). Fresh 4M GH served as the blank. Blank values were subtracted from experimental values to eliminate background readings.

Analytical assays

Commercially available assay kits (collagen and glycosaminoglycan [GAG] assay kits, Biocolor, Newtownabbey, Northern Ireland) were used for the measurement of collagen and sulfated GAGs within the cultured cells, as previously described.[30]

Briefly, for the GAG assay, GAG was extracted from the cultured cells using a solvent system of 4M guanidine-HCl, 0.5M sodium acetate, pH 6, with 1 mM benzamidine-HCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 10 mM N-ethylmaleimide (NEM). Incubation was carried out at 4°C on an orbital shaker for a 12- to 20-h period. After the extraction, the samples were centrifuged, and blyscan dye reagent (composed of 1,9-dimethyl methylene blue in an organic buffer) was mixed with the supernatant. The GAG–dye complex was collected by centrifugation. The dye bound to the pellet was subsequently solubilized by mixing it with a dissociation reagent. The absorbance of the samples was measured at a wavelength of 656 nm, using a UV spectrophotometer. A calibration solution containing chondroitin-4 sulfate was used to obtain the standard curve for this experiment.

The total collagen concentration (acid- and pepsin-soluble fractions) of the cultured chondrocytes was also measured. The acid-soluble collagen was removed by adding 0.5M acetic acid to the cultured cells, followed by centrifugation. The remaining pepsin-soluble collagen was subsequently extracted from the cultured cells. A pepsin solution (1 mg/10 mg tissue sample; Sigma) was added to the cells, and they were incubated overnight at 37°C. Both the acid- and pepsin-soluble collagen samples were further separated for assay by mixing with Sircol dye reagent for 30 min in a mechanical shaker, and the collagen–dye complex was collected by centrifugation. The dye bound to the collagen pellet was solubilized with an alkaline reagent, and the absorbance of the samples was measured at a wavelength of 540 nm, using a UV spectrophotometer. A calibration standard of acid-soluble type I collagen was used to obtain the standard curve for this experiment.

Real-time polymerase chain reaction

To detect the presence of collagen type II and aggrecan, single-stranded cDNA was prepared from 1 μg of total RNA by reverse transcription (RT), using a commercially available First-Strand cDNA kit (Amersham Pharmacia Biotech, Uppsala, Sweden). Subsequently, real-time polymerase chain reaction (PCR) was done using a LightCycler system with LightCycler FastStart DNA Master SYBR Green I

(Roche Diagnostics, Penzberg, Germany). The LightCy-cler™- Primer set (Roche Diagnostics) was used for quantitative detection of the collagen type II and aggrecan genes, and also for quantitation of a housekeeping gene, Glyceraldehide-3-phosphate dehydrogenase (GAPDH), according to the manufacturer's instructions. An initial denaturation step at 95°C for 10 min was followed by amplification and extension steps for 35 cycles (95°C for 10 s, 68°C for 10 s, 72°C for 16 s) with final extension step at 58°C for 10 s. The quantification data were analyzed with the LightCycler analysis software (Roche Diagnostics).

Statistical study

Student's t tests were used to assess whether differences observed between the polymers treated and the control samples were statistically significant. For comparison of groups of means, one-way analysis of variance was carried out. When significant differences were found, Tukey's pairwise comparisons were used to investigate the nature of the difference. Statistical significance was accepted at $p < 0.05$. Values were presented as the mean $\pm$ SD (standard deviation) except in figure 3. Four samples were run for each case. All experiments were repeated at least twice, and similar results were obtained.

RESULTS

Cell proliferation

Chondrocyte proliferation was quantified by crystal violet staining and expressed as a percentage of the
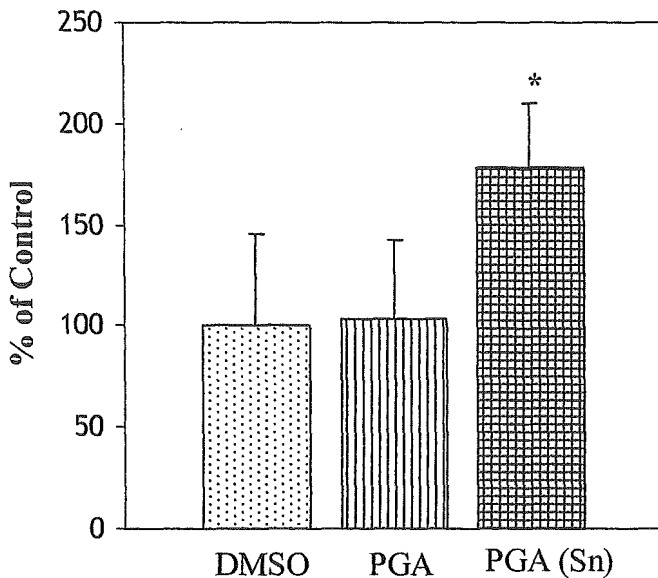

Figure 1. Proliferation of HAC estimated by crystal violet staining. Cell proliferation was significantly increased in PGA(Sn)-cultured chondrocytes compared with that of the control. *$p < 0.05$. All experiments were run in quadruplicate for two separate times.
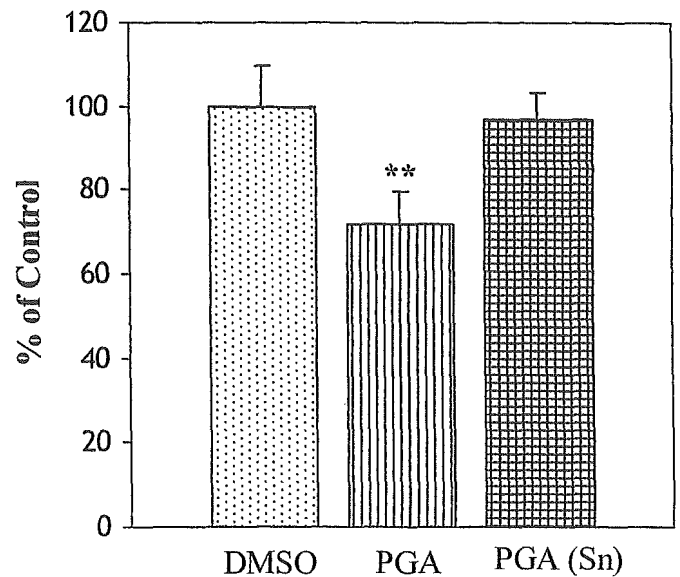

Figure 2. Differentiation of HAC estimated by alcian blue method. Cell differentiation was significantly inhibited in PGA-cultured chondrocytes compared with that of the control. **$p < 0.01$. All experiments were run in quadruplicate for two separate times.

average control value (Fig. 1). Cell proliferation was increased 1.8-fold ($p < 0.05$) in PGA(Sn)-treated cultures compared with that of the control culture, whereas cell proliferation in PGA-treated cultures was almost identical to the DMSO-treated control culture.

Cell differentiation

Chondrocyte differentiation was estimated by alcian blue staining and the amounts were expressed as a percentage of the average control value, which was calculated as 100%. Chondrocytes treated with PGA revealed a 0.71-fold ($p < 0.01$) decrease in cell differentiation compared with that of the control culture. At the same time, cultures treated with PGA(Sn) showed a slight, but nonsignificant, decrease in cell differentiation (Fig. 2).

Extracellular matrix gene expression

Extracellular matrix gene expression was quantitatively measured by real-time PCR. Here, compared with that of the control culture, the collagen type II gene was more strongly expressed ($p < 0.01$) in PGA(Sn) than in PGA-treated cultured chondrocytes [Fig. 3(A)]. Aggrecan gene expression was inhibited in the latter, but no difference was observed between the former and the control culture [Fig. 3(B)].
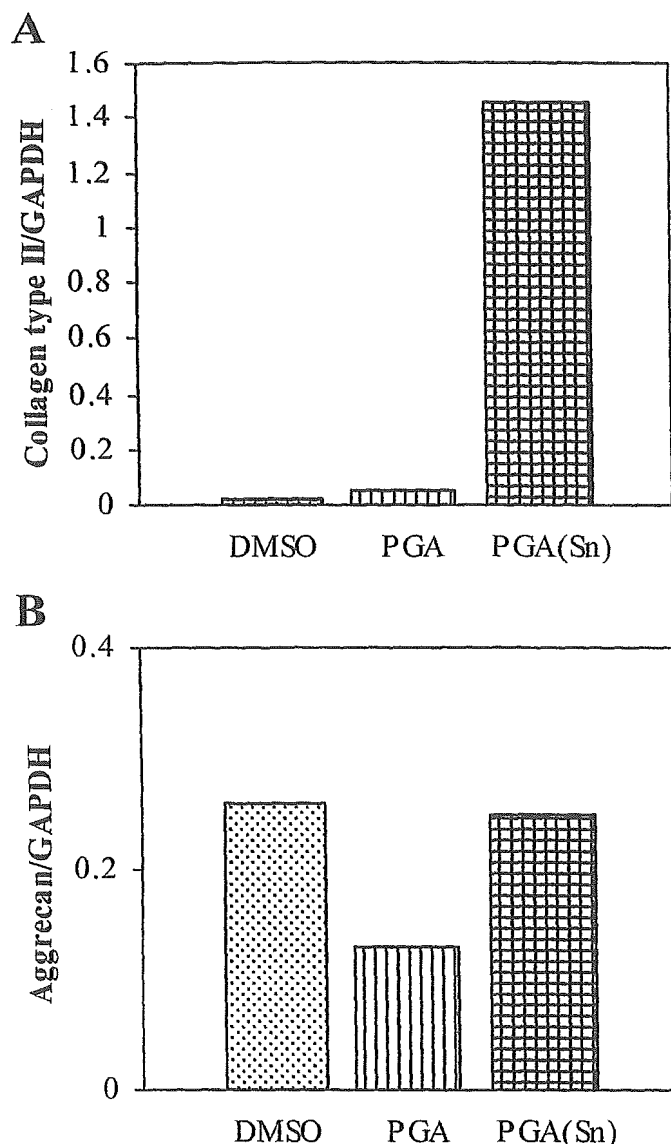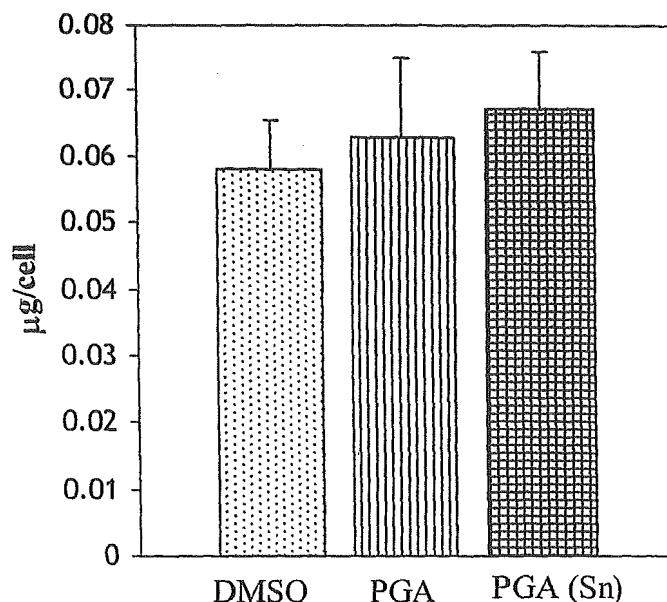
Figure 4. Measurement of collagen type II protein. The amount of collagen type II was increased in PGA(Sn)-treated chondrocytes compared with that of control. All experiments were run in quadruplicate for two separate times.

(Fig. 5). Simultaneously, there was a slight increase in the amount of total collagen in PGA-treated cultures compared with that of the control sample.

Estimation of sulfated glycosaminoglycan concentration

Evaluation of the amount of sulfated GAG showed a decrease in PGA(Sn)-treated cultured cells com-

Figure 3. Extracellular matrix gene expression of HAC by real-time PCR. (A) Collagen type II gene was more strongly expressed in PGA(Sn)- than PGA-cultured chondrocytes compared with that of the control culture. (B) Aggrecan gene expression was inhibited in PGA, but no difference was observed between the PGA(Sn) and the control. All experiments were run in quadruplicate for two separate times.

Measurement of collagen type II protein

The amount of pepsin-soluble and cartilage-specific collagen type II protein was increased in both PGA and PGA(Sn) treated chondrocytes on comparing with that of the control culture(Fig. 4). However, this increase was more in the latter than in the former case.

Measurement of total collagen

Quantitative estimations of both acid- and pepsin-soluble total collagen revealed a decrease in PGA(Sn)-treated cultures compared with that of the control
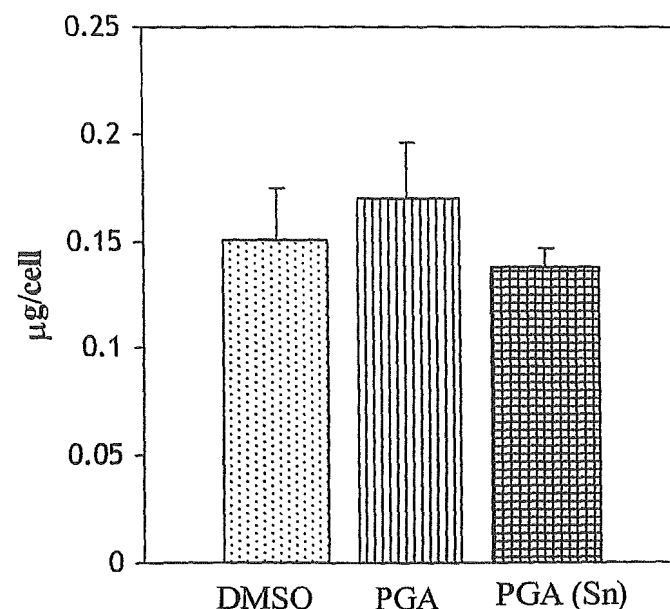
Figure 5. Quantitative estimation of total collagen protein. The amount of total collagen was decreased in PGA(Sn)-treated cultures compared with that of the control. All experiments were run in quadruplicate for two separate times.
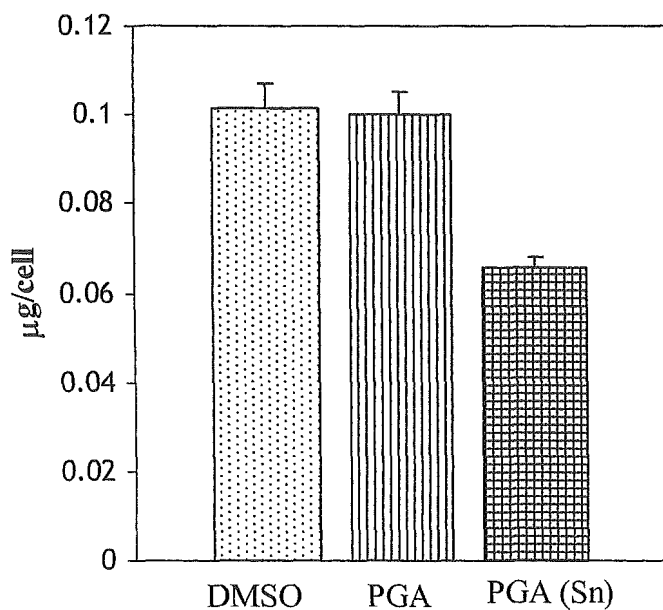
Figure 6. Evaluation of sulfated GAG. There was decrease in the amount of sulfated GAG in PGA(Sn)-treated cultured cells compared with that of the control. All experiments were run in quadruplicate for two separate times.

pared with that of the control (Fig. 6). However, in the same experiment, almost no difference in this amount was observed between the PGA-treated culture and the control.

DISCUSSION

Attempts to identify a perfectly biocompatible and biodegradable polymer have been ongoing over the past decade. An ideal biomaterial should fulfill its purpose satisfactorily and then biodegrade to obviate any risk of foreign body reaction.[31] Synthetic biodegradable polymers, especially those belonging to the polyester family, have played an important role in a number of tissue engineering efforts. PGA, an aliphatic polyester, can be degraded in two ways: by hydrolysis and by nonspecific esterases and carboxypeptidases, followed by either excretion in the urine or entrance into the tricarboxylic acid cycle.[32]

Several different catalysts, namely organotin, antimony, zinc, and lead, are used in the polymerization process to synthesize high molecular weight PGA. Different tin compounds were observed to produce general cytotoxic effects in rabbit articular cartilage in monolayer culture,[33] and bone is suggested to be the critical organ in inorganic tin toxicity in rats.[26] Therefore, in this study, we aspired to evaluate the chondrogenic effects of HAC with PGA synthesized with and without an inorganic tin catalyst, with the aim of clarifying the biocompatibility of inorganic tin as a catalyst for future clinical use.

It was reported that oral administration of certai tin compounds at specific concentrations exerted stin ulatory effects on chondrocyte proliferation in th rat.[33] Consistent with this, the proliferation assay pe formed in our study also showed that HAC wit PGA(Sn) had stimulatory effects on chondrocyte pr liferation in micromass culture (Fig. 1). On the oth hand, PGA neither stimulated nor inhibited the cho drocyte proliferation, and thus, inorganic tin as cat lyst seemed to play a stimulatory role in HAC proli eration. In our experiment, PGA with inorganic tin the catalyst caused almost no change in cell differe tiation, but PGA-treated cultures did show a signif cant decrease when compared with that of the contr (Fig. 2). Furthermore, quantitative estimation of extr cellular matrix gene expression by real-time PCR co firmed that the cartilage-specific protein, collagen ty II, was more strongly expressed in PGA(Sn)- than i PGA-treated cultured chondrocytes [Fig. 3(A)]. Hov ever, the expression of the aggrecan gene was inhit ited in the PGA culture, but no difference was ot served between the PGA(Sn) and the control culture [Fig. 3(B)].

It was reported that oral administration of inorgani tin caused a decrease in the proliferation of chondr cytes, accompanied by suppression of DNA synthesi with subsequent inhibition in collagen synthesis i rat.[34] On the contrary, our results showed enhanc ment of proliferation, expression of the collagen typ II gene, and amount of collagen type II protein by i vitro culture of HAC with PGA(Sn). We speculate that difference in the route of administration might b the cause of these diverse effects of inorganic tin com pound. As mentioned earlier, monolayer culture o rabbit articular cartilage with tin compounds cause inhibition in the synthesis of core proteins, followe by a decrease in the synthesis of sulfated GAG.[33] I agreement with this result, our report also showed decrease in the amount of sulfated GAG by culture c HAC with PGA(Sn). A study performed in our labc ratory using HAC in a micromass culture system ha already shown that PGA synthesized with organic ti catalyst caused a decrease in cell proliferation, but significant increase in cell differentiation [29] and wa completely contradictory to our present results. Th molecular weight of PGA(Sn), and the type of ti product such as SnCl₂ and dibutyl tin were thought t be the key factor of different effects of chondrogenesi on HAC.

To the best of our knowledge, no other study has ye investigated the chondrogenic effects of PGA witl inorganic tin as a catalyst, using HAC in a micromas culture system. This study is the first to show th biological action of inorganic tin as catalyst in PGA o human articular chondrogenesis in a micromass cul ture system. Our observation revealed that low con centration of inorganic tin when used in the polyme

of PGA showed enhancing effects of tin compounds on chondrocytes in comparison to without tin polymer because of increase in the permeability of inorganic tin under the presence of PGA. However, further study is required for the application of this PGA(Sn) in clinical practice.

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ナノレベルイメージングによる分子構造と機能の解析

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井上裕康　　中村　俊　　土屋利江

ナノテクノロジーの臨床　ナノメディシン

ナノレベルイメージングによる分子構造と機能の解析

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Nano-level imaging for analyzing protein structure and function
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Abstract

The present manuscript outlines the nano-level imaging project, which is under promotion by the three national research institutes and supported by a research grant from the Ministry of Health, Labor and Welfare(nano-001). This research project targets collecting fundamental information regarding comprehensive understanding of cardio-vascular, neurological and the other disorders, developing new diagnostic and therapeutic methods by visualizing protein structure and function in atomic(sub-nano level) or molecular(nano-level) resolution. The results of the current projects will be extended into drug design, clinical diagnostic technology and medical materials in near future.

Key words: nano-technology, structural biology, drug design, protein crystallography, tailor-made medicine

は じ め に

21世紀の医療の社会的課題として提唱されているテーラーメード医療の達成には，標的となる蛋白の構造を患者ごとに確定し(分子診断)，最適な薬剤の構造を選択し(分子治療)，薬剤と生体蛋白の相互作用を分子レベルで観察する(分子評価)などの医療基盤技術の育成が求められる．ナノレベルイメージングプロジェクトでは，蛋白分子の構造と機能の解析を通じてテー
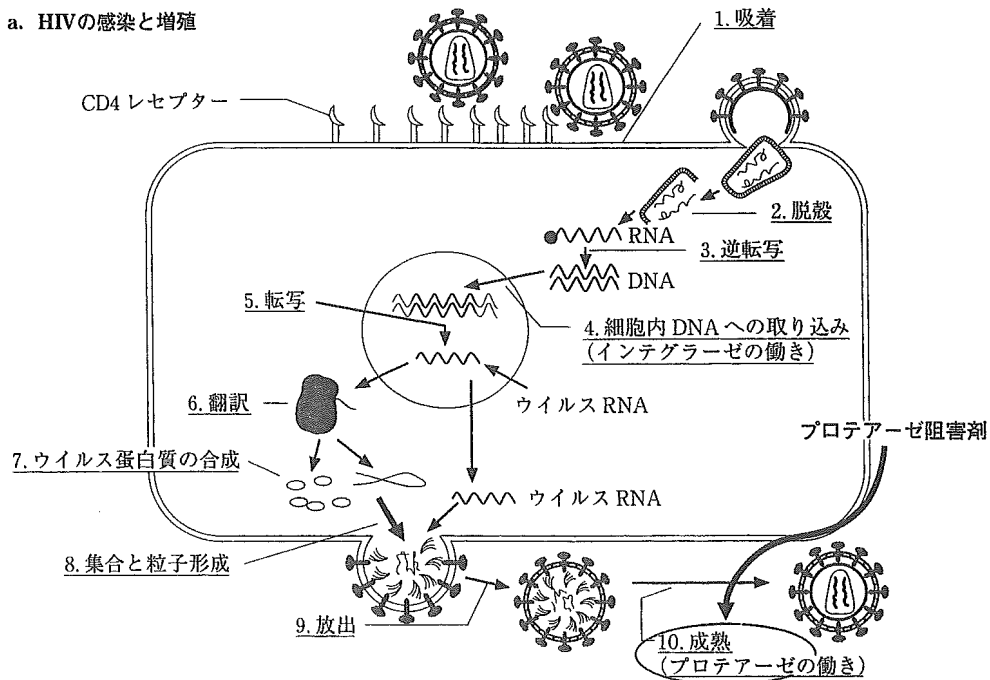
ラーメード医療実現のための基盤技術の形成を目指している．

本稿では蛋白構造イメージングを中心に概説する．

1. 創薬に貢献した分子構造イメージング

近年，放射光を用いたX線回折法の発達により原子レベルの解像度で蛋白結晶の構造を決定できるようになった．構造に基づく薬剤設計の具体的な成功例として，AIDS治療薬(HIVプロ

a. HIVの感染と増殖

1. 吸着

CD4 レセプター

2. 脱殻

RNA

3. 逆転写

DNA

5. 転写

4. 細胞内 DNA への取り込み
（インテグラーゼの働き）

ウイルス RNA

6. 翻訳

プロテアーゼ阻害剤

7. ウイルス蛋白質の合成

ウイルス RNA

8. 集合と粒子形成

9. 放出

10. 成熟
（プロテアーゼの働き）

b. HIVプロテアーゼの構造と阻害剤の設計
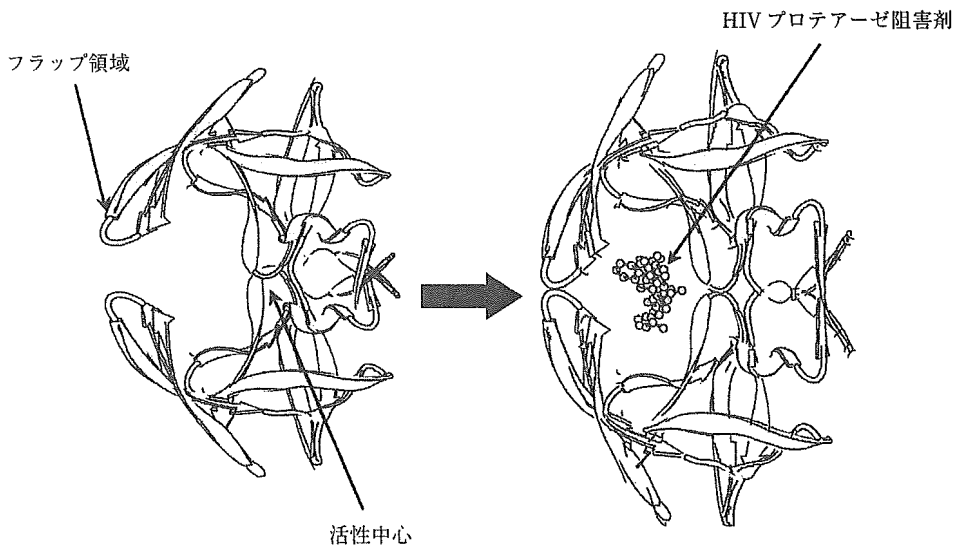
HIV プロテアーゼ阻害剤

フラップ領域

活性中心

図1　AIDS ウイルスの増殖過程と蛋白構造に基づく HIV プロテアーゼ阻害薬の作用機構

テアーゼ阻害薬），白血病治療薬（グリベック）について以下に述べる．

　AIDS ウイルス，HIV は活性化外殻蛋白 gp120 により CD4 陽性 T リンパ球に感染し，自己増殖をする．その際自己由来のプロテアーゼによって前駆体蛋白から活性化外殻蛋白を得る（図1-a）．この HIV プロテアーゼの構造に基づいて設計され，その活性中心を選択的に阻害する目的で設計された薬剤が HIV プロテアーゼ阻害薬である（図1-b）．本剤は AIDS の発症を遅らせることに貢献した[1]．
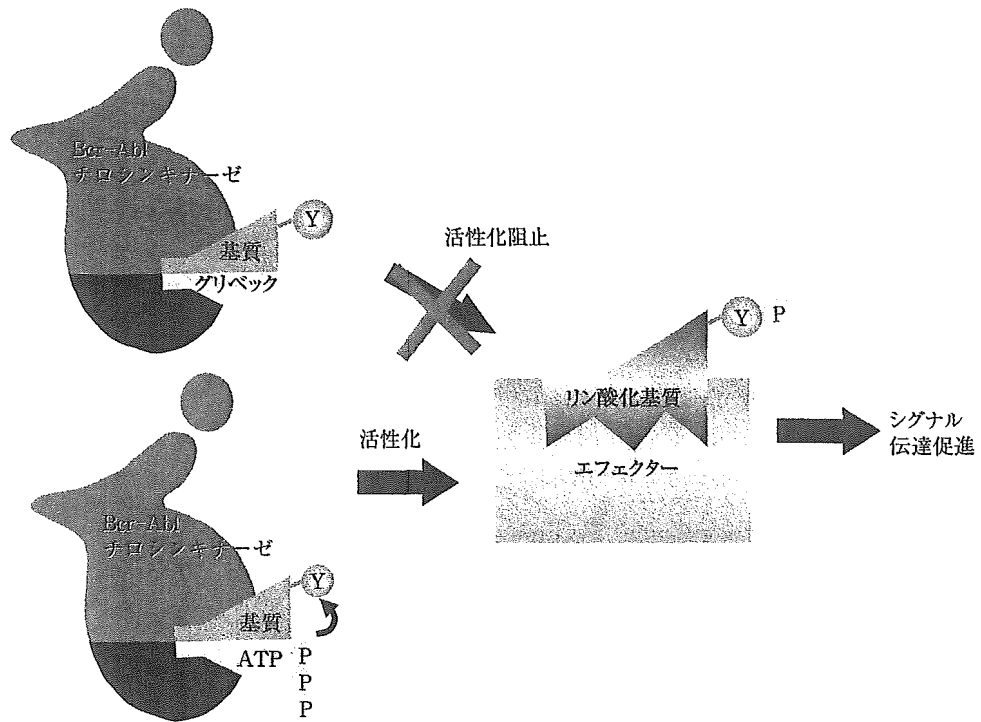
　慢性骨髄性白血病ではフィラデルフィア染色

図2 慢性白血病治療薬(グリベック)の蛋白構造に基づく作用機構

体に由来するBcr-Ablチロシンキナーゼが恒常的な増殖シグナル伝達系の活性化を通じて慢性骨髄性白血病発症の原因になると考えられている. 同酵素はATPと基質に結合し, ATPから切り離したリン酸基で基質のチロシン残基をリン酸化する. グリベックはBcr-AblチロシンキナーゼのATP結合部位の詳細な構造に基づいて設計され, 基質のチロシンリン酸化を構造特異的に阻害して白血病化を防ぐ(図2)[2].

このような構造に基づいて薬剤設計を行うことで標的蛋白との結合の特異性を高め, 副作用を減少させることを期待できる.

2. ヒト心筋トロポニンの構造解析と それに基づく創薬の可能性

心筋収縮を調節する心筋トロポニンの中核部分(コアドメイン)の構造は分担研究者である武田と理化学研究所の前田らによって解析され, Nature誌に報告された(Vol 424, 2003)[3]. 前田らの総説[4]に基づき, トロポニンの筋収縮調節

メカニズムについて述べる.

筋収縮はアクチンとミオシンの滑り運動による. アクチンフィラメントはアクチン, トロポニン, トロポミオシンを含む複合体であり, それらの3分子は7:1:1の存在比をもつ. トロポニンの存在下でアクチンとミオシンはカルシウムイオン濃度に応じた収縮と弛緩を行う.

図3に心筋トロポニンのコアドメインの構造を示す. トロポニンはTnC, TnI, TnTと呼ばれる3つのポリペプチド鎖からなる. これまでの研究により, TnIは収縮抑制因子, TnCは脱抑制因子, TnTはTnCの脱抑制を弱める因子(カルシウム濃度依存性の付加因子)であることが示されている[5].

トロポニンのコアドメインは更に調節頭部とITアームの2つのサブドメインに分かれる. 調節頭部はカルシウムイオンとの結合を通じてトロポニンの構造変化とそれに基づくアクチンとミオシンの滑り運動に対するスイッチの役割を果たす. ITアームは剛性を有するコイルドコイ
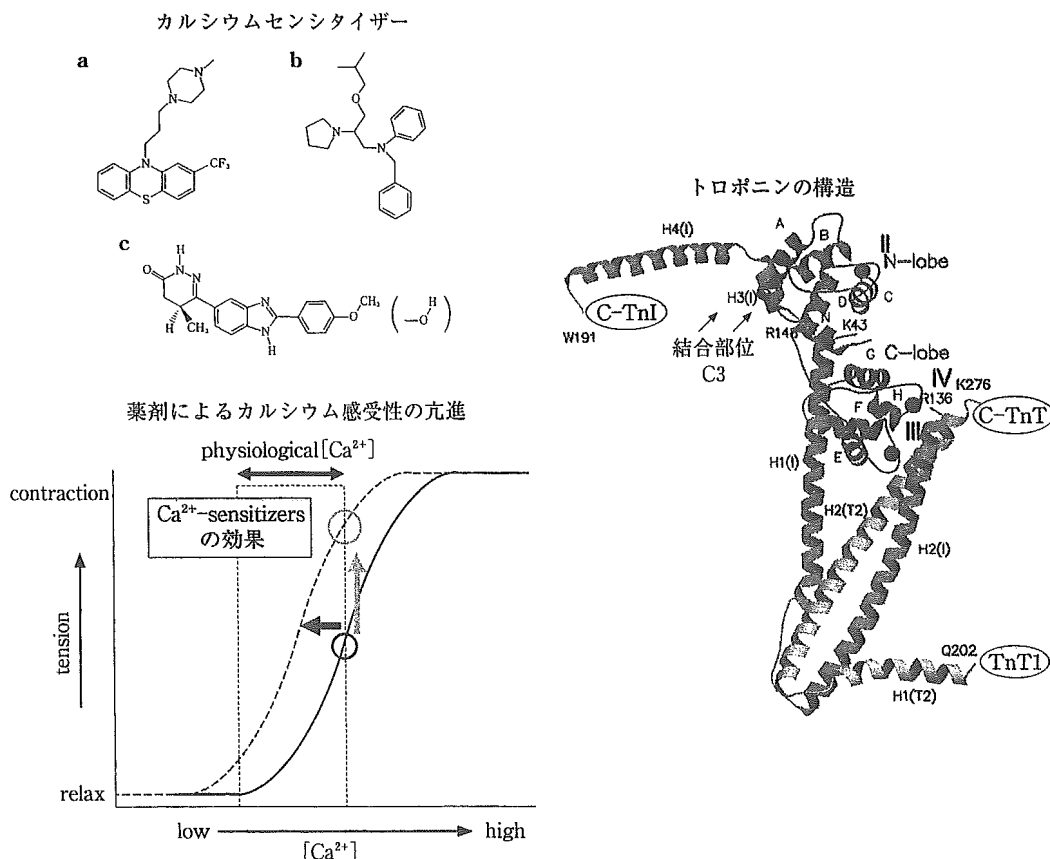
カルシウムセンシタイザー

a　　　b

c

薬剤によるカルシウム感受性の亢進

physiological[Ca²⁺]

contraction

Ca²⁺-sensitizers
の効果

tension

relax

low ──────→ high

[Ca²⁺]

トロポニンの構造

図3　トロポニンコアドメインの構造（文献³⁾より改変引用）

ル構造からなる．TnC は N 末端側と C 末端側の2つの球状部が α ヘリックスで連結された構造をもつ．カルシウム濃度にかかわらず C 末側球状部は TnI に結合し，TnC をトロポニン分子内に常につなぎとめている．一方，TnC の N 末端側球状部は細胞内カルシウム濃度が上昇した場合のみ構造が開き，TnI の第二結合部位（両親媒性 α ヘリックス H3）を結合する．これにより，TnI の調節領域全体がトロポミオシン／アクチンより解離し，アクチンとミオシンの滑り運動が始まる．

TnC の N 末端側球状部にカルシウムセンシタイザーが結合すると，同球状部は開いた構造をとり TnI の第二結合部位を結合しやすくなる．すなわち，TnC による TnI の脱抑制が起こりやすくなる．前述のように TnT は TnC の脱抑制作用にカルシウム濃度依存性を付加することが

できるので，TnC と TnT の制御を組み合わせることで段階的な筋収縮の増強を実現できるかもしれない．近年循環器領域では血管作動性薬剤で優れた新薬が数多く開発されてきたが，ジギタリス以来，これを超える強心剤が生まれていない．従来の強心剤は細胞内カルシウムイオン濃度を高めて強心作用を誘導するために，細胞に対する負荷（カルシウム overload）が不可避であった．1980 年代後半に開発されたカルシウムセンシタイザーと呼ばれた薬剤群はカルシウムイオン濃度−張力関係を左方にシフトさせることにより，低い細胞内カルシウムイオン濃度で高い収縮力を得ることができる理想的な強心剤ではないかと期待された⁶⁾．しかしながら，これらの薬剤の臨床使用経験から，短期的に心筋収縮力は高まるものの，心不全患者の長期予後の改善に役立つことはなかった．これらのカ
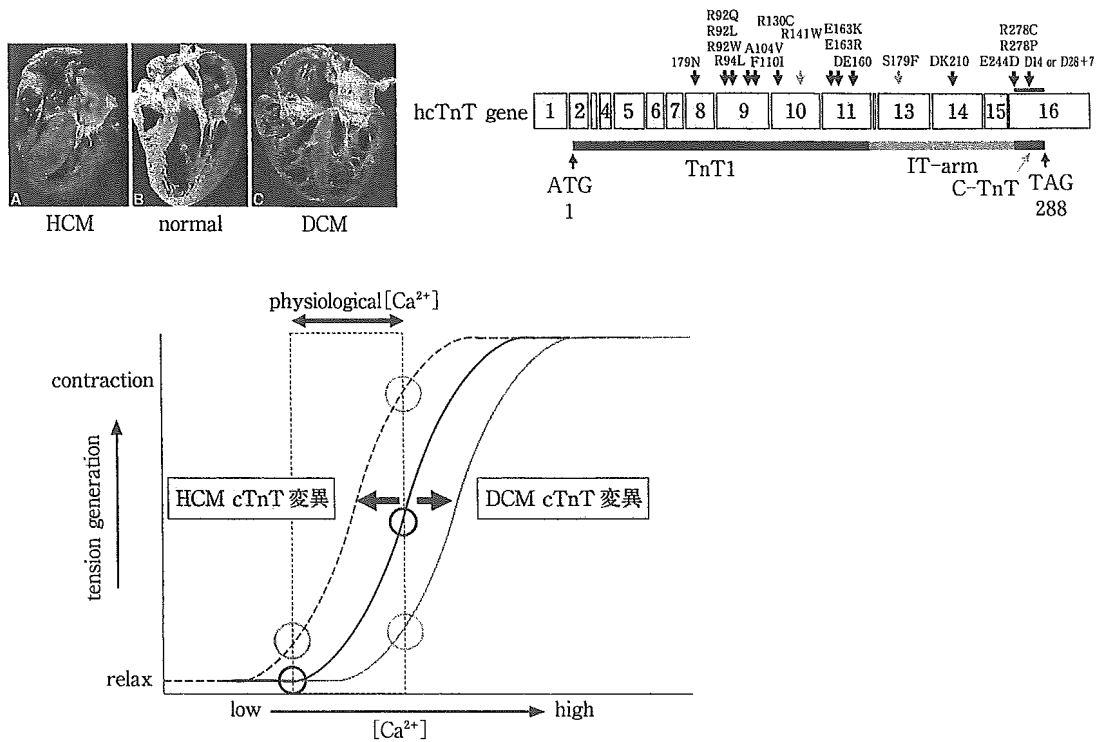
図4　心筋症におけるトロポニンの遺伝子変異と筋カルシウム感受性
心筋症の遺伝子変異はTnT1，C-TnTに多く，筋カルシウム感受性を修飾する.

ルシウムセンシタイザーはphoshodiesteraseの阻害作用も併せてもっており，細胞内cyclic-AMPの増加によって筋小胞体からのカルシウムイオン放出が増加し，ついにはカルシウムoverloadとなる可能性や[7]，構造が類似した他の蛋白と相互作用があるなど，薬剤としての標的特異性が低いことが原因として考えられる.拡張型心筋症例では，少なくとも一部の症例でカルシウム感受性の低下と収縮不全の関連が示唆されている．これらの事実はTnCやTnTを特異的に制御する化合物の設計により，新たな強心剤の開発の可能性を示している.

　一方，肥大型心筋症（HCM）ではトロポニンの遺伝子変異によりカルシウム感受性が亢進することが発病に関連する可能性が示唆されている．同患者の遺伝子解析によると，約15％の患者にTnTの遺伝子変異が認められる．大槻らによれば[5]トロポニンがアクチン/トロポミオシンと直接接触する部分（TnT1，C-TnT，TnI

調節領域）に変異が多く認められ，コアドメインには変異は少ないという（図4）．変異TnTの交換導入を行った心筋スキンドファイバーを用いた研究で，カルシウムイオン濃度-張力関係の左方シフト，すなわちカルシウム感受性の亢進が認められた．この結果からTnTの変異により，カルシウム感受性が亢進し，収縮増加と弛緩不全という肥大型心筋症に特有の症状が発症するという有力な仮説が生まれる．TnTの変異によるカルシウム感受性亢進のメカニズムを原子構造で解明すると，肥大型心筋症に特異的に作用する薬剤の設計を期待できる．原因となる遺伝子変異ごとに構造が異なる薬剤設計が求められる可能性もある．言い換えれば，心筋トロポニンの変異に基づく肥大型心筋症の治療法の開発はテーラーメード医療のモデルケースとなる可能性がある.

細胞膜(リン脂質)

ホスホリパーゼ群

5-LO
FLAP

アラキドン酸 ────→ 種々の細胞 LT 類
(ロイコトリエン)

シクロオキシゲナーゼ
(COX-1 と COX-2)

アスピリン
インドメタシン

PGH_2

合成酵素群

mPGES

血小板ほか　肥満細胞　種々の細胞　血管内皮　胎盤ほか
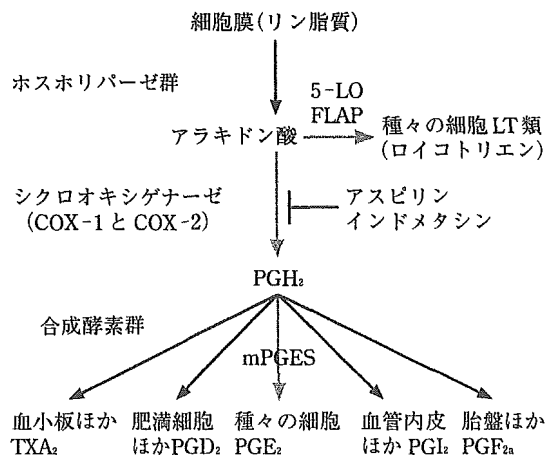TXA_2　　ほかPGD_2　PGE_2　ほか PGI_2　PGF_{2a}

図5　プロスタグランジン産生系

3. 創薬の標的として注目されている プロスタグランジン合成酵素群の 構造解析

シクロオキシゲナーゼ(COX)はプロスタグランジン(PG)を生合成する律速酵素として知られている(図5)2種類のアイソザイムが存在する．COX-1 は constitutive enzyme と呼ばれ，ほとんどの細胞で常時発現しており，生体の安定性を維持する役割を果たす．一方，COX-2 は inducible enzyme として，単球，線維芽細胞，滑膜細胞などの炎症にかかわる細胞で発現し，炎症性サイトカインなどによって誘導される．従来の非ステロイド系抗炎症剤は，COX-1 と COX-2 の両方を阻害するために炎症巣の PG だけでなく，胃粘膜や腎での PG (特に PGE_2)産生を抑制し胃や腎の副作用を合併する．そこで，炎症に深く関与していると考えられる COX-2 だけを選択的に阻害する薬剤の開発が進められてきた．このようにして開発された COX-2 阻害薬は胃潰瘍を起こしにくい鎮痛剤として好んで投薬されていた．しかしながら，2004 年末，米政府は，これらの COX-2 選択的阻害薬の3剤を心筋梗塞や脳梗塞の危険性を高める恐れがあるとして，心臓病患者への処方や多量の長期使用を避けるよう勧告した．COX-2 の下流に位置するプロスタサイクリン合成酵素の作用も

抑制するために，同酵素に由来する抗血栓性作用や血流増加作用が損なわれることが原因ではないかと考えられている[8]．図5に示したように COX-2 の下流には多くの合成酵素があってそれぞれの作用を有する蛋白を合成している．個々の合成酵素を選択的に阻害する薬剤の開発が次世代の創薬の標的として注目される．PGE_2 の産生にかかわる mPGES を阻害する薬物の開発は血管内血栓形成を伴わない理想的な抗炎症剤となる可能性がある．TXA_2 産生を阻害する薬剤の開発は血管内血栓形成の予防，局所血流増加作用を通じて脳梗塞，心筋梗塞の予防薬や治療薬として期待できる．PGI_2 は既に難病といわれた原発性肺高血圧症の治療に有効であることが知られている．PG 関連薬剤の開発は構造に基づく創薬の最大の標的の一つになっており，ナノメディシンプロジェクトでも複数の関連酵素の構造解析に取り組んでいる．

4. ナノメディシンプロジェクトの そのほかの研究

本プロジェクトでは分子構造イメージングに関連して上記のほかに，細胞内イオン環境や，血管新生にかかわる蛋白など幾つかの蛋白構造についても研究を進めている(国立循環器病センター研究所)．国立精神神経センターでは in-silico スクリーニング法による Parkinson 病の治療薬探索に蛋白構造情報を応用する研究を進めている．国立医薬品食品衛生研究所では原子間力顕微鏡を用いて蛋白表面の詳細な構造を解析することなどを通じて，医用材料作成に向けた応用研究に取り組んでいる．

一方，分子機能イメージングの領域では，国立循環器病センターの望月らが増殖因子(EGF)刺激に伴う Ras 分子の活性化を FRET 法で可視化できることを Nature 誌に報告した[9]．ナノメディシンプロジェクト開始後も血管内皮の走化運動にかかわる Rap1 蛋白の可視化に関する研究などに FRET 法による分子イメージングを展開している．国立精神神経センターの研究グループでは分子機能イメージング技術を応用してシナプス機能，プリオン蛋白質の機能の評価に

取り組み Proc Natl Acad Sci などの雑誌に研究成果を報告している[10].

お わ り に

本ナノメディシンプロジェクトでは循環器治療の中核施設である国立循環器病センター内に構造生物学ラボを立ち上げ，分子特異的な治療薬の開発を目指している．ナノ DDS 技術や分子機能イメージング技術に関する研究を併せて推進することで，特異的分子治療薬の分子輸送技術開発と他の分子との相互作用の可視化技術を推進することが可能となる．これにより，分子診断・分子治療・分子評価を包含するテーラーメード医療の基盤形成に貢献したい.

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