寄附講座事業成果報告書

部局名：徳島大学大学院医歯薬学研究部

寄附講座等の名称：心臓血管病態医学分野（設置期間：平成 23 年 1 月 1 日～令和 2 年 12 月 31 日）

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１　寄附講座等の概要

生活習慣病、メタボリックシンドローム等により心臓病、動脈硬化性脈管疾患が発症する機序を明らかにして、新規の診断、治療技術を開発する。循環器内科学分野と連携して、臨床で得られる血液、白血球、手術標本、遺伝子を解析して、データベースを構築し、病態を解析する。診断技術、治療法を考案し、モデル動物を用いて基礎研究、前臨床試験を行う。循環器内科によって行われる臨床試験への橋渡しを行う。薬物，運動療法，食事療法などの治療介入が心臓血管疾患に及ぼす効果を評価し，その機序を探求する。これらの研究を通して循環器内科学の発展に貢献する。

研究内容
(1) 動脈硬化の病態解明，新規制御因子の同定
(2) 抗動脈硬化薬物療法の開発
(3) 動脈硬化イメージング技術の開発
(4) 動脈硬化のバイオマーカーの開発
(5) 肥満が心機能，血管機能に及ぼす影響の検討
(6) 心臓血管疾患の病態における脂肪組織の役割に関する研究

２　教育・研究・診療等の成果・効果

生活習慣病によって慢性炎症が惹起される新しい機序の解明と治療法の開発をめざし、数々の基礎研究、臨床研究の成果を発表した。

１．肥満に伴う脂肪組織における慢性炎症の機序に関する研究

（１）肥満に伴う脂肪細動の変性と cfDNA の増加

マウスに高脂肪食(High Fat Diet, HFD)を投与すると、肥大化脂肪細胞が細胞死を起こしていた。また、肥満によって cfDNA としての single strand (ss) DNA, double strand (ds) DNA の濃度が上昇した（図 1 上段）。ヒトにおいても内臓脂肪の蓄積に伴って血中の ssDNA 濃度が上昇した（図 1 下段）。

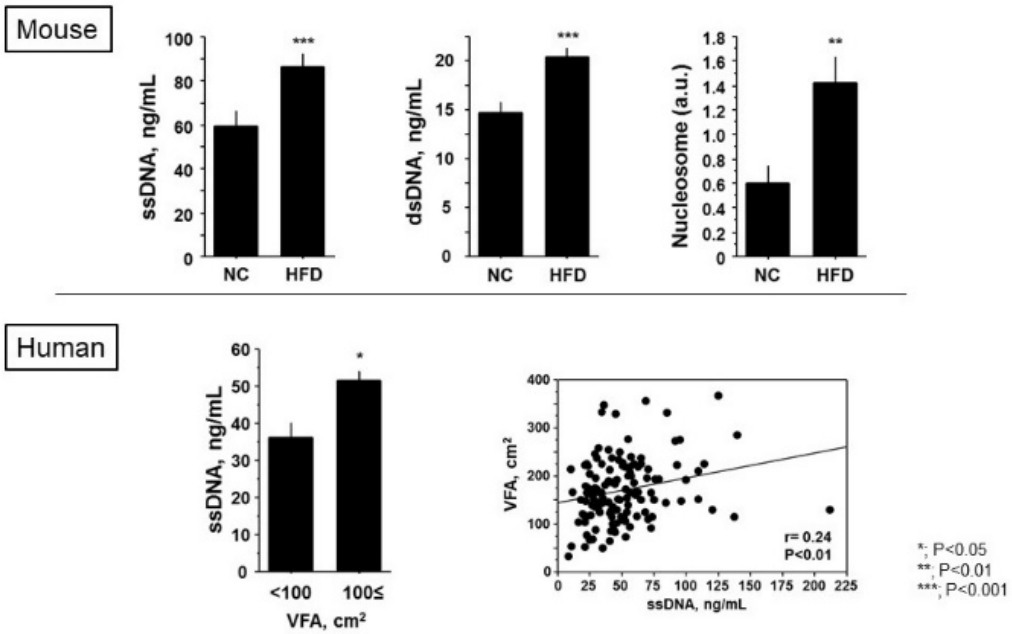

図 1. 肥満にともない、cfDNA 濃度が上昇する

（上段）マウスに高脂肪食負荷(HFD）を投与すると、血中の ssDNA, dsDNA, nucleosome の濃度が上昇した。（下段） ヒトにおいても内臓脂肪面積（VFA）と血中 cfDNA 濃度は相関し、インスリン抵抗性と関連した。

（2） *TLR9* 欠損マウスにおける脂肪組織における炎症の減弱とインスリン抵抗性の減弱

野生型マウスと *TLR9* 欠損マウスに高脂肪食(HFD)を投与したところ、*TLR9* 欠損マウスでは、肥満に伴う脂肪組織へのマクロファージの浸潤とインスリン抵抗性が軽減していた（図 2）。また、骨髄移植の実験によって、骨髄由来細胞に発現している *TLR9* が、肥満に伴う、脂肪組織での炎症とインスリン抵抗性の発現に重要であることが明らかになった。

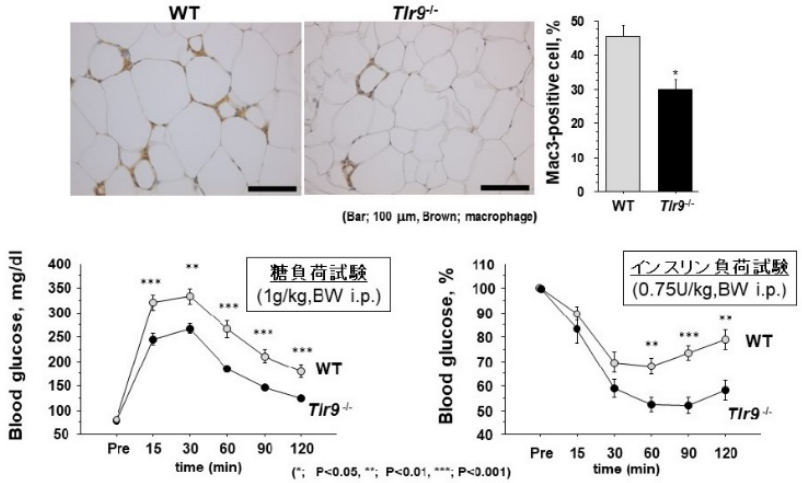

図 2. 肥満に伴う脂肪組織における慢性炎症とインスリン抵抗性に関して、野生型と *TLR9* 欠損マウスの比較

（上段）*TLR9* 欠損マウスにおいては、野生型マウスに比較して、肥満に伴うマクロファージの浸潤が軽減していた。（下段） *TLR9* 欠損マウスにおいては、肥満に伴うインスリン抵抗性が軽減していた。

２．脂肪細胞由来の cfDNA がマクロファージを活性化する機序の検討

3T3-1 脂肪細胞培養液の上清を、野生型ならびに *TLR-9* 欠損マウス由来のマクロファージに添加すると、炎症性サイトカインの産生が増加した。脂肪細胞を TNFα で刺激するとその程度は増加した。また、*TLR9* 欠損マウス由来のマクロファージでは軽減していた（図 3）。

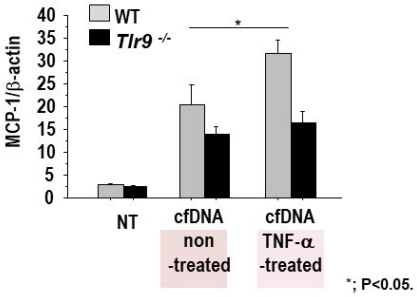

図 3. 3T3L1 脂肪細胞培養上清によるマクロファージの活性化

3T3L1脂肪細胞培養上清中のDNAを濃縮して、野生型ならびに*TLR9*欠損マウス由来のマクロファージに添加したところ、MCP-1の産生が亢進した。脂肪細胞をTNF-αで傷害しておくとその程度が増強した。脂肪細胞培養上清によるマクロファージ活性化作用は、*TLR9*欠損マウス由来のマクロファージでは減弱していた。

３．動脈硬化における慢性炎症の機序に関する研究

生活習慣病によってどのようにして慢性炎症が惹起されるのかを検討した。肥満に伴って脂肪組織から自己由来の遊離核酸断片 (cell free DNA, cfDNA)が放出され、本来、病原体由来の DNA 断片を認識する Toll 様受容体-9 (*TLR9*)を cfDNA が活性化し、脂肪組織での慢性炎症ならびにインスリン抵抗性が引き起こされた。

また、アポリポ蛋白 E 欠損（*ApoE* KO）マウスに Angiotensin Ⅱ (Ang Ⅱ)を投与することで、血漿中の 1 本鎖 DNA などの cfDNA が増加した。大動脈において、cfDNA の受容体となりうる TLR9 の発現が Ang Ⅱ投与により有意に増加し、主たる発現細胞はマクロファージであった。*TLR9*/*ApoE 2* 重欠損(dKO)マウスを確立し、Ang Ⅱ投与下で誘導される動脈硬化病変を解析した。両群間で体重、血圧、血漿脂質値に差を認めなかったが、*TLR9*/*ApoE* dKO マウスの動脈硬化病変およびプラーク内脂質量は、*ApoE* KO マウスに比べて有意に少なかった。また、*TLR9*/*ApoE* dKO マウスの腹部大動脈において、MCP-1 などの炎症性物質の発現やマクロファージの浸潤が減少していた。さらに、骨髄移植実験により、血管壁より骨髄の *TLR9* が重要であることが明らかとなった。また、TLR9 のアゴニストや Ang Ⅱなどで傷害を与えた血管内皮細胞を用いてマクロファージを刺激することで、NF-κB シグナルの活性化が認められ、TNF-α や MCP-1 などの炎症関連分子の発現が有意に増加した。

以上より、血管傷害により遊離した cfDNA が、マクロファージ *TLR9* を活性化し血管の炎症を惹起し、動脈硬化の進展を促進することが示唆された。

４．TLR9/cfDNA をターゲットとした生活習慣病の治療法の開発

（１）肥満に伴う脂肪組織の炎症とインスリン抵抗性に対する戦略

マウスに、*TLR9* の阻害作用のあるオリゴヌクレオチドを腹腔内に週３回注射すると、肥満に伴う脂肪組織へのマクロファージ浸潤とインスリン抵抗性が改善した（図 4）。

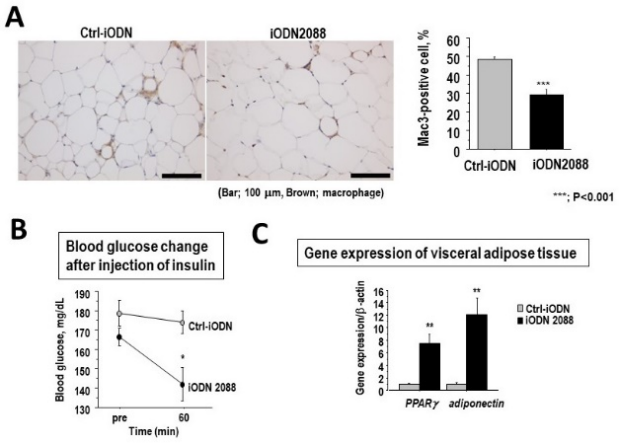

図 4. TLR9 阻害オリゴヌクレオチドによる炎症抑制効果

マウスの腹腔内に*TLR9*阻害オリゴヌクレオチドiODN2088を投与したところ、肥満に伴う脂肪細胞の炎症が抑制された（A）。また、iOD2088の投与は、インスリン抵抗性を改善して（B）、脂肪組織でのインスリン感受性改善遺伝子の発現を増強した（C）。

（2）肥満に伴う脂肪組織の炎症とインスリン抵抗性に対する戦略

ApoE 欠損マウスに、*TLR9* の阻害作用のあるオリゴヌクレオチドを腹腔内に週３回注射すると、動脈硬化性病変の進行が抑制された。また、動脈硬化プラーク内へのマクロファージの浸潤が減弱し、脂肪蓄積が抑制された。また、炎症性サイトカインや MMP9 の発現が抑制された。

以上の結果をもって 31 名（うち 6 名は留学生）の大学院生が、学位を取得した。

生活習慣病による慢性炎症が惹起される機序の解明に大きく貢献することができた。その成果は、米国、ブラジル、ロシア、日本の一般紙で市民に大きく報道された。また、NHK ためしてガッテン、シブ５時、あさイチ、日本テレビ　世界一受けたい授業、テレビ朝日　林修の今でしょ、たけしの家庭の医学などで広く報道された。

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海外招待講演

1. Masataka Sata “Epicardial adipose tissue plays a role in the pathogenesis of Atherosclerosis” APSC Subspecialty Congress-Intervention and Imaging 2012, Taipei, Taiwan, May 4 to 6, 2012.
2. Masataka Sata “Inflammation in atherosclerosis and adipose tissue” The 12th Biennial International Endotoxin & Innate Immunity Society (IEIIS) Meeting 2012, Tokyo, Japan, October 23 to 26, 2012.
3. Masataka Sata “Novel insights into the pathogenesis of atherosclerosis : epicardial adipose tissue vasa vasorum, and anti-aging genes” 2014 “Metabosartan” Expert Summit, Taipei, Taiwan, March 16, 2014.
4. Masataka Sata “Epicardial adipose tissue plays a role in the pathogenesis of atherosclerosis” The 18th International Vascular Biology Meeting, Kyoto Japan, April 14 to17, 2014.
5. Masataka Sata “Novel Insights into the Pathogenesis of Atheroclerosis : Epicardial Adipose Tissue, Vasa Vasorum, and Anti-aging Genes”2014 International Forum of Hypertension, Atherosclerosis and Metabolic Syndrome, Taipei, Taiwan, May 25th, 2014
6. Masataka Sata “The role of NOAC in atherosclerotic disease” The Annual Scientific Meeting of Taiwan Society of Lipids & Atherosclerosis 2015 and The 15th Taipei International Vascular Biology Symposium, Taipei, Taiwan, September 12-13, 2015
7. Masataka Sata “The role of innate immunity in the pathogenesis of atherosclerosis” The Annual Scientific Meeting of Taiwan Society of Lipids & Atherosclerosis 2015 and The 15th Taipei International Vascular Biology Symposium, Taipei, Taiwan, September 12-13, 2015
8. Masataka Sata “How do you treat AF patients with CAD and what does the evidence tell us?” The Annual Scientific Meeting of Taiwan Society of Lipids & Atherosclerosis 2015 and The 15th Taipei International Vascular Biology Symposium, Taipei, Taiwan, September 12-13, 2015
9. Masataka Sata “The Functional Implication of Novel Inflammatory Mediators on the Atherogenesis and Metabolic Derangement” The 59th Annual Scientific Meeting of The Korean Society of Cardiology, Goyang, Korea, October 16-17, 2015
10. Masataka Sata. “Role of innate immunity in chronic inflammation in arterial wall and adipose tissue.” 19th International Vascular Biology Meeting, Boston, America, October 30-November 3, 2016
11. Sata M. Role of epicardial fat in cardiovascular disease. ESC2018,Munich,August 25-28,2018
12. Sata M, SGL T2 and CHF, The 49th Annual Convention & Scientific Session of the Taiwan Society of Cardiology, Taippei, Taiwan, May, 19, 2019

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３　達成度（自己評価）

この十年間で、当初の目標をほぼ達成できたと考える。

４　その他