南野直人、友池仁暢：高中性脂肪血症ウサギの内臓脂肪組織および血清のプロテオーム解析、日本動脈硬化学会第38回総会、一般演題（2006年７月東京）

3. Harada-Shiba M, Minamino N, Kuwahara H, Ito T, Maeda R, Ohira M, Abe E, Jinno K, Tomoike H: Proteome analysis of hypertriglyceridemic rabbits. XIV International symposium on Atherosclerosis (Rome,Italy, June 18-22, 2006 - Roma Marriott Park Hotel)

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

厚生労働科学研究費補助金（健康科学総合研究事業）
分担研究報告書

食後高トリグリセリド血症（PHT）家兎におけるＴＧ分解に関する基礎的検討

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研究要旨

ヒト生活習慣病のモデル動物の開発を目的として我々が開発したウサギ（食後高トリグリセリド血症家兎（PHT; Postprandial Hyper Triglyceridemia rabbit）の中性脂肪（トリグリセリド；TG）が食後に増加する原因について，TG の分解酵素であるリポ蛋白リパーゼ（LPL）と肝性トリグリセリドリパーゼ（HTGL）の蛋白量を測定する事により検討した。PHT のヘパリン投与前の LPL は対照（日本白色家兎；JW）との間に差異を認めなかったが，ヘパリン投与後の LPL は，対照に比較して有意に低い値を認めた。同様に HTGL を測定した結果，PHT の HTGL はヘパリン投与前及び投与後共に，対照との間に差異を認めなかった。

血中 TG の増加の原因として，肝臓からの TG の分泌亢進，TG の代謝（異化）の低下，血中 TG の肝臓への取り込みの障害等，種々報告されている。今回の結果から，PHT の食後 TG 増加は，TG 分解酵素である LPL 量が不足しているために TG が分解されずに血中に残存した事が原因の一つと考えられた。

A. 研究目的

リポ蛋白リパーゼ（LPL）は食事由来の代謝経路により小腸で合成されるカイロミクロン(CM)や内因性の代謝経路により肝臓で合成される超低比重リポ蛋白（VLDL）のトリグリセリド（TG）を加水分解する酵素である。また，肝性トリグリセリドリパーゼ（HTGL）は LPL と類似の構造と機能を持つ酵素であり，主にレムナントリポ蛋白を分解する。LPL 及び HTGL の機能低下は高 TG 血症の原因の一つと考えられている。今回我々は，食後高トリグリセリド(TG)血症家兎（PHT; Postprandial Hyper Triglyceridemia rabbit）の食後 TG 増加の原因を検討するために，TG 分解酵素である LPL と HTGL の酵素量を測定し，対照と比較検討した。

B. 研究方法

供試動物は 10 カ月齢の雄性の PHT 及び日本白色家兎（JW）が各 6 匹である。これらの動物にヘパリン（500 U/0.5ml/Kg）を静脈投与し，

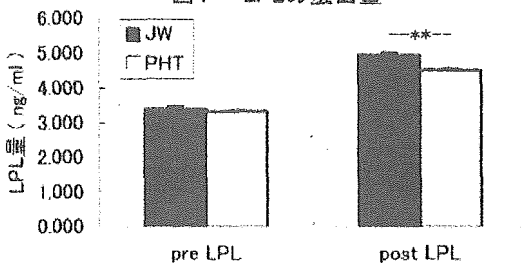
投与前と投与 10～15 分後の血漿を用い LPL と HTGL を測定した。LPL 及び HTGL の測定にはそれぞれ市販の測定キットである LPL エライザ「第一」(第一化学薬品(株)) 及び HTGL ELISA マルピー (大日本製薬 (株)) を用いた。

(倫理面への配慮) 実験動物に際しては、動物愛護上の配慮を充分に行い、当研究施設の実験動物委員会の指針に基づいて行った。

C. 研究結果

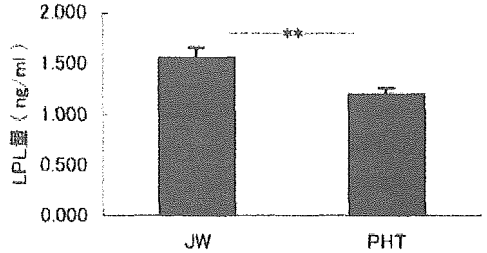
LPL蛋白量についての測定結果を図 1 に示す。ヘパリン投与前の LPL は PHT (3.319±0.037 ng/ml:平均値±標準誤差) と JW (3.397±0.076 ng/ml) に差異を認めなかったが, ヘパリン投与後の PHT (4.506±0.037 ng/ml) は, JW (4.950±0.083 ng/ml) に対し, 有意に低い値を示した。ヘパリン投与前と後の差である Delta LPL を求めたところ, PHT (1.186±0.064 ng/ml) は, JW (1.553±0.105 ng/ml) に比較して有意に低い値を示した (図 2)。

図1 LPLの蛋白量



Mean±S.E.M ** p<0.01 Student's T-Test

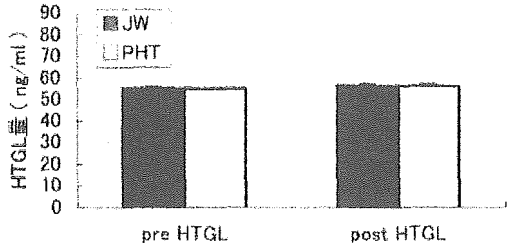
図2 Delta LPL



Mean±S.E.M ** p<0.01 Student's T-Test

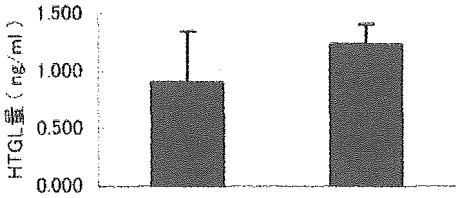
一方, 同様にHTGLの蛋白量を測定した結果, ヘパリン投与前の PHT (54.982±0.219 ng/ml) と JW (55.234±0.244 ng/ml) 並びにヘパリン投与後の PHT (56.214±0.315 ng/ml) と JW (56.130±0.572 ng/ml) の間に差異を認めなかった (図 3)。Delta HTGL も同様に, PHT (1.231±0.172 ng/ml) と JW (0.895±0.446 ng/ml) との間に差異を認めなかった (図 4)。

図3 HTGLの蛋白量



Mean±S.E.M

図4 Delta HTGL



Mean±S.E.M

D. 考察

PHTの食後TG増加の原因を検討するために, TG 分解酵素である LPL と HTGL を測定した。その結果PHTにLPL蛋白量の有意な低値を認めた。LPL はカイロミクロンや VLDL 中の TG を加水分解する酵素であるため, PHT の血中 TG 増加は, LPL の蛋白量不足が原因の一つと考えられる。しかし, これら酵素の本来の働きを検討するためには, LPL と HTGL の活性を測定する

必要がある。さらに，今回は，TGの代謝（異化）を中心に検討したが，血中TG増加の要因には，肝臓からのTGの過剰放出や肝臓へのリポ蛋白の取り込み不足等も考えられるため，今後検討を加えたい。

E．結論

PHTはTGの分解酵素であるLPLの蛋白量が不足していた。したがって，PHTの食後TG増加はLPLの不足が原因の一つと考えられた。

F．研究発表

1．論文発表

1. Ito T, Ohwada K, Tomoike H. A hereditary postprandial hypertriglyceridemic(PHT) rabbit model.

Nippon Yakurigaku Zasshi.

2005; 125(5): 301-306

2. Tasaki K, Wakabayashi I, Shishido T, Takasaki S, Takeishi Y, Kubota I, Ito T, Katano Y, Tomoike H. Diminution of angiotensin Ⅱ-induced contraction of abdominal aorta isolat-ed from Watanabe heritable hyperlipidemic rabbits.

J. Smooth Muscle Res.

2005; 41(2):87-97

3. Shimoda T, Ishihata A, Aita T, Kaga M, Ito T, Ohwada K, Tomoike H, Katano Y. Progression of severe atherosclerosis amd increased arterial pulse pressure in the newly developed heritable mixed hy-perlipidaemic rabbits.

Clin Exp Pharmacol Physiol.

2006; 33(3):221-226

2．学会発表

1. 下田智子，會田智美，利　美賀子，友池仁暢，伊藤恒賢，大和田一雄，石幡　明，片野由美：遺伝性高中性脂肪血症家兎における循環動態および加齢変化（Effects of aging and elevated level of plasma triglyceride on blood），(2005)，第78回日本薬理学会年会講演要旨集、182-182（2005年3月22日横浜）

2. 伊藤恒賢：食後高トリグリセリド血症家兎の開発と有用性，(2005)，日本実験動物技術者協会東北支部，特別講演（2005年4月9日福島市）

3. 伊藤恒賢，秦　正充，三ツ口陽子，大和田一雄，友池仁暢：食後高トリグリセリド血症（PHT）家兎におけるＴＧ産生と分解に関する基礎的検討，(2005)，第52回日本実験動物学会総会、122-122（2005年5月20日東京）

4. 三ツ口陽子，伊藤恒賢，大和田一雄：当施設で開発した遺伝性高ＴＧ血症家兎(TGH)の病理組織学的基本特性の検索，(2005)，第52回日本実験動物学会総会、188-188（2005年5月20日東京）

5. Tsunekata Ito, Kazuo Ohwada, Hitonobu Tomoike: A hereditary post-prandial hypertriglyceridemic (PHT) rabbit model. The 1st International Conference on Transgenic Rabbits, Tsukuba, Japan, June 18, 8-8, 2005

6. 伊藤恒賢，秦　正充，三ツ口陽子，大和田一雄，斯波真理子，友池仁暢：食後高トリグリセリド血症家兎（PHT）の平時における血中脂質とインスリン動態，(2005)，第39回日本実験動物技術者協会総会、113-113（2005年6月25日金沢市）

7. 秦　正充，伊藤恒賢，大和田一雄，斯波真理子，友池仁暢：食後高トリグリセリド血症家兎(PHT)の食後におけるアポ蛋白プロファイル，(2005)，第39回日本実験動物技術者協会総会、114-114（2005年6月25日金沢市）

8. 三ツ口陽子，伊藤恒賢，大和田一雄，斯波真理子，友池仁暢：食後高トリグリセリド血症家兎(PHT)の基本的病理組織像の特性検索，(2005)，

第39回日本実験動物技術者協会総会、110-110
（2005年6月25日金沢市）

G. 知的財産権の出願・登録状況

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厚生労働科学研究費補助金（健康科学総合研究事業）
分担研究報告書

低 HDL 血症に関わる新規遺伝子の検索と機能解析
ABCA1 およびその近縁関連遺伝子の機能と反応機構

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研究要旨

1）ABCA1 遺伝子の発現制御が、細胞内遊離コレステロールレベルの制御機構である ACAT の阻害剤と PPARa アゴニストのフィブラートが、LXA/RXR を介して行われること、カルシウムチャネル拮抗剤が LXR/RXR とは独立に ABCA1 遺伝子の転写を促進することを証明した。2）ABCA1 のカルパイン分解による翻訳後制御がアポリポ蛋白質刺激による燐酸化により調節されること、プロブコールが ABCA1 の機能とカルパイン分解を阻害することを示した。3）ABCA1 の相同遺伝子 ABCA7 は、強制発現系に於いてコレステロールの少ない小粒子 HDL のみを産生することを示した。ABCA7 は SREBP2 により ABCA1 とは逆方向の制御を受けることを見いだした（投稿中）。4）ABCA1 ノックアウトマウスの系が確立し、LCAT-KO マウスとの交配による細胞コレステロール搬出機構の二重欠損の病態解析を開始した。

A. 研究目的

末梢細胞はコレステロールを異化することが出来ず、細胞内ステロール代謝平衡を維持するためには、細胞外に搬出されねばならない。動脈硬化巣の細胞に蓄積したコレステロールを減少させるためには、或いは蓄積を積極的に防ぐには、この細胞内コレステロールの搬出システムに頼ることになる。このためのコレステロールの自由拡散と並ぶ主要な機構が、アポリポ蛋白質と細胞の ABCA1 蛋白質の相互作用による HDL の新生反応である。そして、ABCA1 の機能不全は低 HDL 血症の主要な原因の一つであることが明らかにされつつある。本研究の目的はこの反応の機序を解明し、反応の賦活化とそれによる低 HDL 血症の改善により動脈硬化症予防の手段とすることである。

B. 研究方法

1）この反応の細胞側の主要因子である ABCA1 遺伝子の転写制御機構を検討した。2）ABCA1 活性のカルパイン分解による翻訳後制御の機序を検討した。3）ABCA1-KO マウスを、背景遺伝子を C57Bl/6 に統一して確立、LCAT-KO マウスとの交配で二重欠損マウス系を確立した。

4）ABCA1 と ABCA7 による HDL 新生反応の詳細を検討し、ABCA7 の遺伝子プロモータ解析と発現調節機構を行った。

（倫理面への配慮）実験動物に際しては、動物愛護上の配慮を充分に行い、当研究施設の実験動物委員会の指針に基づいて行った。

C and D. 研究結果と考察

1）ABCA1 転写の促進

ABCA1 は細胞内コレステロール量を検知して転写レベルで発現増加が起こる。この結果コレステ

ロールエステル化酵素ACAT の阻害による ABCA1 の発現増加を検証した。ACAT 阻害剤に加え、ACAT 遺伝子の欠損によっても ABCA1 遺伝子の活性化が起こることを明らかにした (Sugimoto et al. BBA 1636: 69, 2004; Yamauchi et al. JLR 45; 1943, 2004)。臨床的に抗高脂血症剤として広く用いられているフィブラートによる HDL の上昇が ABCA1 の発現調節の直接関与を検討した。ABCA1 の転写と蛋白質の増加が認められ、レポーターアッセイによりLXR に依存する反応と確認された (Suzuki et al, ATVB 25: 1193, 2005)。また、臨床的に HDL 上昇が報告されているカルシウムチャネル拮抗剤について、ABCA1 の転写促進が認められ、これが LXR/RXR 系に依存しないことを確かめた (ATVB 24: 519, 2004)。

2) 我々は先に ABCA1 の翻訳後活性制御がカルパインによる分解行われ、アポリポ蛋白質がこれを抑制する調節が行われていることを見いだした。これの反応について、ApoA-I による HDL 新生反応に於けるスフィンゴミエリン搬出の補充反応から生じるジグリセリドが、PKCa 活性化を介してABCA1 の燐酸化を行うことを示した (Yamauchi et al, JBC 278: 47890, 2003)。またこれは両親媒性ヘリックスペプチドに広く認められる機能であることも示した (Arakawa et al., JBC 279: 6217, 2004)。さらに、ABCA1 の阻害剤であるプロブコールが、ABCA1 を形質膜状で不活性化し、カルパイン分解に対しても抵抗性を与えることを明らかにした Wu et al, JBC 279: 30168, 2004)。

3) ABCA1 の生理的役割を検討するために、欠損マウスの確立を図った。Jakson Laboratory から DBA マウスを遺伝子背景にもつ ABCA1-KO マウスを得、我々の他の遺伝子改変マウスとの対照実験を容易にするためその遺伝子背景を系代交配により C57Bl/6 に変更を完了した。これと非特異的細胞コレステロール搬出系の障害を持つ LCAT-KO マウスを交配し、ABCA1/LCAT 二重欠損マウスを確立して、詳細を検討中である (投稿準備中)。

4) ABCA1 の相同遺伝子 ABCA7 の機能を解析した。ABCA7 は強制発現細胞に於いて ABCA1 と同様 HDL 新生反応を媒介できる (Abe-Dohmae et al, JBC 279: 604, 2004)。この反応の詳細を ApoA-I と A-II による HDL 新生で検討し、ABCA1 はコレステロールに富んだ直径 13 nm の大型の HDL とコレステロールに乏しい直径 10 nm の小型の HDL が産生するが、ABCA7 によっては後者しか産生されず、ABCA1 が細胞コレステロール搬出に於いてより効率的な機構を備えていることが分かった (Hayashi et al, JLR 46: 1703, 2005)。また血漿アミロイド蛋白質 A (SAA) による HDL 新生を検討し、他のアポリポ蛋白質と同様の HDL 新生能を有することが明らかになった (論文投稿中)。さらに ABCA7 のプロモータ解析を詳細に行い、この遺伝子が SREBP2 により negative 調節を受け、細胞ステロールによる制御は ABCA1 と逆方向であること、細胞の呑食作用と密接に関わる機能を有していることを示した (論文投稿中)

E. 結論

ABCA1 とその近縁関連遺伝子の発現調節機能と反応機構を研究し、その HDL 新生に於ける役割を明らかにした。

F.研究発表

1. 論文発表

1. Tsujita M, Cheng-Ai Wu, Abe-Dohmae S, Usui S, Okazaki M, Yokoyama S. On the Hepatic Mechanism of HDL Assembly by the ABCA1/ApoA-I Pathway.

J. Lipid Res. 2005; 46: 154-162

2. Ito J, Nagayasu Y, Rui Lu, Alireza Kheirollah,

Hayashi M, Yokoyama S . Astrocytes produce and secrete fibroblast growth factor-1 which promotes production of apoE-high density lipoprotein in a manner of autocrine action.

J. Lipid Res. 2005; 46: 679-989

3. Arakawa R, Tamehiro N, Nishimaki-Mogami T, Ueda K , Yokoyama S. Fenofibric acid, an active form of fenofibrate, increases apoAI-mediated HDL biogenesis by enhancing transcription of ABCA1 gene in an LXR-dependent manner.

Arterioscl. Thromb. Vasc. Biol. 2005; 25: 1193-1197

4. Yokoyama S. Assembly of high density lipoprotein by the ABCA1/apolipoprotein pathway,

Current Opinion in Lipidology. 2005; 16: 269-279

5. Hayashi M, Abe-Dohmae S, Okazaki M, Ueda K, Yokoyama S. Heterogeneity of high density lipoprotein generated by ABCA1 and ABCA7

J. Lipid Res. 2005; 46: 1703-1711

6. Yokoyama S. Assembly of high density lipoprotein. ***Arteriosclr. Thromb. Vasc. Biol. 2006; 26: 20-27***

7. Dohmae S, Ueda K, Yokoyama S. ABCA7, a molecule with unknown function.

FEBS Letters. in press

8. Ito J, Alireza Kheirollah, Nagayasu Y, Rui Lu, Kato K, Yokoyama S. Apolipoprotein A-I increases association of cytosolic cholesterol and caveolin-1 with microtubule-cytoskeletons in rat astrocytes. ***J. Neurochem. in press***

2. 学会発表

1. 斯波真理子、山本章、横山信治、都島基夫、山村卓、高木敦子、吉政康直、友池仁暢 ; 家族性高コレステロール血症と autosomal recessive hypercholesterolemia の臨床像、第 37 回日本動脈硬化学会、シンポジウム（2005 年 7 月 14-15 日東京）

2. 辻田麻紀、奥平桂一郎、呉成愛、堂前純子、横山信治：血管壁内細胞からのアポリポ蛋白質による特異的脂質搬出機構、第 37 回日本動脈硬化学会、シンポジウム（2005 年 7 月 14-15 日東京）

3. 岩本紀之、堂前純子、胡魏、林弥智、植田和光、横山信治：ABCA1-KO 及び野生型マウスにおける ABCA7 発現分布の解析、第 37 回日本動脈硬化学会、一般演題（2005 年 7 月 14-15 日東京）

4. Anwar Hossain、辻田麻紀、横山信治：LCAT/ABCA1 欠損マウスにおける細胞コレステロールの恒常性の検討、第 37 回日本動脈硬化学会、一般演題（2005 年 7 月 14-15 日東京）

5. 為広紀正、佐藤陽治、浅川義範、橋本敏弘、大野泰雄、井上和秀、横山信治、最上知子：HDL 新生を促進する LXR□選択的アゴニスト Riccardin C の発見、第 37 回日本動脈硬化学会、一般演題（2005 年 7 月 14-15 日東京）

6. 辻田麻紀、鵜川眞也、Anwar HOssain、島田昌一、横山信治：低 HDL 血症マウス舌におけるコレステロール受容体の検討、第 37 回日本動脈硬化学会、一般演題（2005 年 7 月 14-15 日東京）

7. 堂前純子、胡魏、石神秀昭、岩本紀之、公文義雄、岡崎三代、呉成愛、辻田麻紀、植田和光、横山信治：SAA による HDL 新生反応、第 37 回日本動脈硬化学会、一般演題（2005 年 7 月 14-15 日東京）

8. 胡魏、堂前純子、辻田麻紀、公文義雄、横山信治：SAA 含有 HDL 産生メカニズムと ABCA1、第 37 回日本動脈硬化学会、一般演題（2005 年 7 月 14-15 日東京）

9. 林弥智、堂前純子、岡崎三代、植田和光、横山信治：Heterogeneity of HDL Generated by ABCA1 and ABCA7、第 37 回日本動脈硬化学会、一般演題（2005 年 7 月 14-15 日東京）

10. 荒川礼二郎、為広紀正、最上知子、植田和光、横山信治：Fenofibrate の HDL 上昇作用に関する検討ー細胞内 ABCA1 発現に及ぼす作用、第 37 回日本動脈硬化学会、一般演題（2005 年 7 月 14-15 日東京）

11. 伊藤仁一、長安祐子、Alireza Kheirollah、横山信治：Apolipoprotein A-I Increased Association of Cytosolic Cholesterol with Microtubules in Rat Astrocytes、第 48 回日本神経化学会、ミニシンポジウム（2005 年 9 月 28-30 日、福岡）

12. 辻田麻紀、鵜川眞也、Anwar Hossain、島田昌一、横山信治：低コレステロール血症マウ

ス味蕾細胞でのコレステロール受容体の検討、第78回日本生化学会、一般演題(2005年10月13-16日、神戸)

13. 伊藤仁一、長安祐子、Alireza Kheirollah、呂鋭、横山信治；アポリポ蛋白質A-I誘導性細胞内コレステロール輸送促進に於ける微小管の役割、第78回日本生化学会、一般演題（2005年10月13-16日、神戸）

14. Alireza Kheirollah、伊藤仁一、長安祐子、横山信治：ApoA-I-Induced Intracellular Cholesterol Trafficking is suppressed by CsA Through Interaction with PP2B、第78回日本生化学会、一般演題（2005年10月13-16日、神戸）

15. 呂鋭、伊藤仁一、横山信治：Apolipoprotein A-I Stimulates Tubulin Phosphorylation by Cytosolic Caveolin-1 and Microtubule-Associated Protein Kinase C、第78回日本生化学会、一般演題（2005年10月13-16日、神戸）

16. 堂前純子、胡魏、石神秀昭、岩本紀之、公文義雄、岡崎三代、呉成愛、辻田麻紀、植田和光、横山信治：Serum Amyloid AによるABCA1/ABCA7依存性HDL新生、第78回日本生化学会、一般演題（2005年10月13-16日、神戸）

17. 為広紀正、河原陽介、吉田武美、植田和光、横山信治、最上知子：VerapamilによるABCA1遺伝子の活性化機構、第78回日本生化学会、一般演題（2005年10月13-16日、神戸）

18. Anwar Hossain、辻田麻紀、横山信治：ABCA1/LCAT両蛋白欠損マウスのコレステロール平衡の検討、第78回日本生化学会、一般演題（2005年10月13-16日、神戸）

19. 岩本紀之、胡魏、林弥智、植田和光、横山信治：野生型およびABCA1-KOマウスにおけるABCA7の組織発現分布とコレステロール負荷による変化、第78回日本生化学会、一般演題（2005年10月13-16日、神戸）

20. 胡魏、堂前純子、公文義雄、辻田麻紀、横山信治：ABCA1によるSAA含有HDLの産生、第78回日本生化学会、一般演題（2005年10月13-16日、神戸）

21. 西田智、伊藤仁一、長安祐子、横山信治：参加ストレスに対するFGF-1/apoEを介した脳保護機構、第78回日本生化学会、一般演題（2005年10月13-16日、神戸）

22. Maki Tsujita、Kei-ichiro Okuhira、Shinji Yokoyama：Apolipoprotein A-I Dissociates From HDL and Reacts with ABCA1 Protein to Generate HDL Particles、FEBS Special Meeting "ABC2006-ATP Binding Cassette (ABC) Proteins: From Multi Drug Resistance to Genetic Disease"、Oral Session（2006年3月4-10日、Innsbruck）

23. Noriyuki Iwamoto、Sumiko Abe-Dohmae、Ryuichiro Sato、Shinji Yokoyama：ATP-Binding Cassette Transporter A7 is Negatively Regualted by Cholesterol and Needed to Promote Phagocytosis through the Sterol Responsive/Regulatory Element Pathway、FEBS Special Meeting "ABC2006-ATP Binding Cassette (ABC) Proteins: From Multi Drug Resistance to Genetic Disease"、Poster Session（2006年3月4-10日、Innsbruck）

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

別紙４

研究成果の刊行に関する一覧表レイアウト

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Mariko Harada-Shiba	Gene Transfer and Target Diseases	K.Taira K.Kataoka T.Niidome	Non-viral Gene Therapy	Springer	Tokyo	2005	246-260

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Akira Yamamoto Mariko Harada-Shiba , Michinori Endo, Norio Kusakabe, Tsuneo Tanioka, Hiroshi Kato, Takashi Shoji	The effect of ezetimibe on serum lipids and lipoproteins in patients with homozygous familial hypercholesterolemia undergoing LDL-apheresis therapy	Atherosclerosis			in Press
Tsunekata Ito, Kazuo Ohwada, Hitonobu Tomoike	A hereditary Postprandial Hypertriglyceridemic (PHT) Rabbit Model	Folia Pharmacol Jpn	125	301-306	2005
Katsunari Tasaki, Ichiro Wakabayashi, Tetsuro Shishido, Satoshi Takasaki, Yasuchika Takeishi, Isao Kubota, Tsunekata Ito, Yumi Katano, Hitonobu Tomoike	Diminution of Angiotensin Ⅱ-Induced Contraction of Abdominal Aorta Isolated From Watanabe Heritable Hyperlipidemic Rabbits	J. Smooth Muscle Res	41(2)	87-97	2005
Tomoko Shimoda Akira Ishihata Tomomi Aita Mikako Kaga Tsunekata Ito Kazuo Ohwada Hitonobu Tomoike Yumi Katano	Progression of Severe Atherosclerosis and Increased Arterial Pulse Pressure in the Newly Developed Heritable Mixed Hyperlipidemic Rabbits	Clinical and Experimental Pharmacology and Physiology	33	221-226	2006
Reijiro Arakawa, Norimasa Tamehiro, Tomoko Nishimaki-Mogami,	Fenofibric Acid, an Active Form of Fenofibrate, Increases apoAI-mediated HDL Biogenesis by	Arterioscl. Thromb. Vasc. Biol.	25	1193-1197	2005

Kazumitsu Ueda , Shinji Yokoyama	Enhancing Transcription of ABCA1 Gene in an LXR-Dependent Manner				
Michi Hayashi, Sumiko Abe-Dohmae, Mitsuyo Okazaki, Kazumitsu Ueda, Shinji Yokoyama	Heterogeneity of High Density Lipoprotein Generated by ABCA1 and ABCA7.	J. Lipid Res.	46	1703-1711	2005
Jin-ichi Ito, Alireza Kheirollah, Yuko Nagayasu, Rui Lu, Koichi Kato, Shinji Yokoyama	Apolipoprotein A-I Increases Association of Cytosolic Cholesterol and Caveolin-1 with Microtubule-Cytoskeletons in Rat Astrocytes.	J. Neurochem.			in press

Gene Transfer and Target Diseases

Mariko Harada-Shiba

1 Gene Transfer and Target Diseases

The first human gene-transfer protocol was developed in 1989, in an attempt to track lymphocytes in the immunologic treatment of melanoma and renal cell cancer. Although many attempts have been made in clinical trials of gene therapy, the FDA has not approved the marketing of any gene therapy agent.

For the successful therapeutic application of gene therapy, the delivery of several kinds of genes and nucleotides has been tested in animal models of genetic diseases as well as in patients to supply missing proteins to maintain cellular function, or to deliver proteins that induce proliferation or apoptosis of the cell, etc. For example, gene therapy has been applied to treating cardiovascular diseases, including coronary artery disease (CAD), peripheral artery disease (PAD), restenosis after vascular interventions and graft failure, hyperlipidemia, thrombosis, and cancer (e.g., lung, kidney, prostate, brain). In this chapter, the pathophysiology of several diseases and the application of gene delivery in their treatment will be described.

2 Cardiovascular Diseases

2.1 Coronary Artery Disease and Peripheral Artery Disease

Atherosclerosis is the most prevalent process that affects adult coronary and peripheral arteries. Atherosclerotic lesions narrow arteries, leading to a reduction of the arterial blood supply to the myocardium and skeletal muscle. Stimulation of collateral vessel formation by the use of gene therapy will help to increase perfusion of the ischemic tissues. Genes encoding growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and hepatocyte growth factor (HGF),

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have been successfully tested in animal models and clinical trials for therapeutic angiogenesis.

VEGFs are mitogenic and survival factors for endothelial cells, and promote angiogenesis and lymphangiogenesis. The VEGF family consists of six members, VEGF-A, -B, -C, -D, -E and placental growth factor (PLGF), which differ in their molecular masses and biological properties (Dvorak et al. 1995; Leung et al. 1989; Maglione et al. 1993; Joukov et al. 1996; Achen et al. 1998; Ogawa et al. 1998; Olofsson et al. 1996). The three receptors, VEGFR-1, VEGFR-2 and VEGFR-3, have tyrosine kinase activity. VEGF-A is the most well known member of the VEGF family, as it plays a crucial role in angiogenesis and vasculogenesis (Ferrara 2000, 2001). In addition, VEGF-A has several splice variants, of which two ($VEGF_{121}$ and $VEGF_{165}$) have been reported to be angiogenic in both animal models and clinical trials (Losordo et al. 1998; Vale et al. 2000). $VEGF_{121}$ is readily diffusible because it lacks a heparan-sulfate binding site, whereas $VEGF_{165}$ binds to the matrix after being secreted. VEGF-B, -C, -D, -E and PLGF also show angiogenic activity in animal models (Yoon et al. 2003; Rissanen et al. 2003; Kiba et al. 2003).

The FGF family has twenty-three members that share 30%–70% identical amino acid sequences. These growth factors act directly on vascular cells and induce endothelial cell growth and angiogenesis. Among the FGFs, FGF-1, -2, -4 and -5 have been tested for their angiogenic activity in animal models and are also the subjects of clinical trials in the therapy of cardiovascular disease and Atherosclerosis obliterans (ASO) (Ueno et al. 1997b; Javerzat et al. 2002; Grines et al. 2003). FGFs are multifunctional proteins that act through various alternatively spliced isoforms with four tyrosine kinase receptors, FGFR-1, -2, -3 and -4 (Galzie et al. 1997; Ornitz et al. 1996).

HGF is another multifunctional growth factor that stimulates the proliferation and migration of endothelial cells. In rabbit, rat and mouse ischemia models, HGF was reported to stimulate angiogenesis (Morishita et al. 1999, 2002; Morishita 2004; Hayashi et al. 1999). The efficacy and safety of intramuscular injection of naked human HGF plasmid was recently demonstrated in clinical trials in 22 patients with PAD or Berger's disease (Morishita 2004).

Angiopoietins (Angs) are also growth factors for vascular development. The Ang family has four members, Ang-1, -2, -3, -4, all of which bind to Tie-2, a tyrosine kinase receptor (Ward and Dumont 2002). Ang-1/Tie-2 and VEGF/VEGFR2 are crucial for the mobilization and recruitment of hematopoietic stem cells and the recruitment of circulating endothelial progenitor cells (Hattori et al. 2001). Ang-1 decreases the inflammatory response and promotes vascular maturity; thus, a combination of VEGF and Ang-1 may be a good strategy for therapeutic angiogenesis (Siddiqui et al. 2003; Yamauchi et al. 2003).

Hypoxia-inducible transcription factor (HIF)-1α can activate several genes involved in angiogenesis, such as VEGF, VEGFR-2, IGF-2 and erythropoietin (Levy et al. 1995). Adenovirus-mediated HIF-1α gene therapy is currently in clinical testing for the treatment of myocardial ischemia.

2.2 Restenosis After Vascular Interventions and Vein Graft Failure

The occlusion of arteries after balloon angioplasty, stenting, or the failure of bypass vein graft is a major factor that determines the prognosis of peripheral and coronary

artery disease. Smooth muscle cell proliferation, remodeling, matrix deposition, thrombosis, and platelet and leukocyte adhesion may all play a role in the development of arterial restenosis in these settings (Topol and Serruys 1998). In order to decrease vascular cell proliferation, various gene therapy strategies have been employed. Antiproliferative strategies designed for the treatment of experimental cardiovascular disease can be grouped into two main categories: (1) antisense approaches, ribozymes, and transcription-factor decoy strategies to inactivate positive cell cycle regulators; (2) overexpression of negative regulators of cell growth.

Transfection to arterial smooth muscle cells with thymidine kinase combined with gancyclovir, antisense oligonucleotides and ribozymes against cell cycle regulators, c-*myb*, c-*myc*, *cdc*-2, *cdk*-2, *ras*, *bcl*-x, and decoy constructs against transcription factors, such as E2F and NFκB, have all been shown to inhibit neointimal proliferation (Morishita et al. 1993, 1997; Indolfi et al. 1995; Pollman et al. 1998; Burgess et al. 1995; Suzuki et al. 1997). Inhibition of the cell cycle by transfection of genes encoding the non-phosphorylated forms of the retinoblastoma gene products p21, p27, p53, or of the growth arrest homeobox gene (*gax*) has been reported in animal models (Tanner et al. 1998; Yonemitsu et al. 1998; Chang et al. 1995a,b; Smith et al. 1997).

The transfection of genes encoding growth factors, including VEGF and HGF, results in decreased neointima formation in experimental animals (Hiltunen et al. 2000; Laitinen et al. 1997). The rapid regeneration of endothelial cells by growth factors restored the secretion of nitric oxide, C-type natriuretic peptide (CNP) and prostacyclin I_2, which have anti-proliferative effects on smooth muscle cells. The local expression of CNP suppressed neointimal formation in injured arteries of rats and vascular remodeling in porcine coronary arteries (Ueno et al. 1997a; Morishige et al. 2000).

2.3 Hyperlipidemia

Some hyperlipidemias are congenital, caused by a monogenic disorder. The strategies of gene therapy in hyperlipidemia are divided into three groups. (1) to supply the defective gene to correct the dyslipidemia, (2) to overexpress proteins involved in lipid metabolism, and (3) miscellaneous approaches.

Several studies have applied the first therapy strategy to the treatment of monogenic hyperlipidemias. Familial hypercholesterolemia (FH) is caused by defect in the LDL receptor, which results in severe hypercholesterolemia beginning at birth, cutaneous and tendon xanthomas, and atherosclerosis in childhood (Goldstein JL 2001). Trials of LDL or VLDL receptor gene delivery to the liver were carried out in WHHL rabbits and FH patients (Grossman et al. 1994, 1995; Kozarsky et al. 1994; Pakkanen et al. 1999; Lebherz et al. 2004; Kankkonen et al. 2004). The Apo A1 gene encoding a protein necessary for HDL synthesis and involved in reverse cholesterol transport, was delivered in patients deficient in this gene (Benoit et al. 1999). Apo E gene transfer was shown to be successful in treating *apoE* knockout mice (Cioffi et al. 1999; Okamoto et al. 2002; Gough and Raines 2003; Harris et al. 2002). Lipoprotein lipase (LPL), which catalyzes triglyceride formation in chylomicrons and VLDL, were delivered in LPL-deficient animals (Excoffon et al. 1997; Liu et al. 2000). Delivery of the gene encoding lecithin cholesterol acyl transferase (LCAT) corrected dyslipidemia in patients deficient in the gene and in hypoalphalipoproteinemia patients (Brousseau et al. 1998; Seguret-Mace et al. 1996).

The overexpression of proteins involved in the regulation of lipid metabolism has been tested in several systems. Hepatic expression of the catalytic subunit of apolipoprotein B mRNA editing enzyme (Apobec-1) reduced serum LDL-cholesterol levels in normal and WHHL rabbits (Greeve et al. 1996; Kozarsky et al. 1996). Expression of secreted "decoy" human-macrophage scavenger receptors (MSR) inhibited foam-cell formation in murine macrophages (Jalkanen et al. 2003a,b). Overexpression of LPL in transgenic WHHL rabbits improved hyperlipidemia and obesity (Koike et al. 2004). Finally, the long-term expression of human apo A-1 increased HDL size and inhibited atherosclerosis progression in LDLR knockout mice (Belalcazar et al. 2003).

2.4 Thrombosis

Gene transfer has a number of cardiac and systemic applications disease conditions, such as acute coronary syndromes, restenosis following percutaneous coronary intervention (PCI) and venous grafts, and thrombotic states. The strategies for gene therapy of thrombosis are: (1) to inhibit the coagulation pathway or platelet aggregation, (2) to activate fibrinolysis, and (3) to modulate endothelial function.

In order to target the coagulation cascade, transfer of the hirudin gene led to reduced intimal hyperplasia in a rat carotid-artery injury model (Rade et al. 1996). Gene delivery of thrombomodulin, a cell-surface glycoprotein of endothelial cells that binds thrombin, was reported to reduce thrombus formation in a rabbit model of stasis-induced arterial thrombosis (Waugh et al. 1999). In a rabbit carotid shear-stress-induced model of thrombosis, overexpression of the tissue-factor pathway inhibitor (TFPI) gene reduced thrombus formation (Nishida et al. 1999). Overexpression of cyclooxygenase-1, an inhibitor of platelet aggregation, increased production of the antiplatelet prostaglandin prostacyclin and reduced thrombus production in a porcine model of balloon-injury-induced carotid thrombosis (Zoldhelyi et al. 2001). Transfer of the nitric oxide (NO) synthase gene was reported to reduce arterial thrombosis in a rat carotid injury model and in a porcine coronary artery balloon-injury model (von der Leyen et al. 1995).

The targeted activation of fibrinolysis was achieved by overexpressing either recombinant tissue-type plasminogen activator (rTPA) or surface-anchored urokinase in endothelial cells and then seeding these cells into grafts in order to increase fibrinolytic activity. Reduced local platelet and fibrin deposition were observed while systemic markers of coagulation and fibrinolysis remained unchanged (Dichek et al. 1996).

Overexpression of NO synthase modulates endothelial function, inhibiting platelet adhesion after arterial injury (Yan et al. 1996).

2.5 Primary Pulmonary Hypertension

Primary pulmonary hypertension is a rare but life-threatening disease that causes right ventricular failure and death. The average survival from the time of diagnosis is 2.8 years (Nagaya 2004). In order to reduce pulmonary vascular resistance, the transfer of genes encoding eNOS, calcitonin gene-related peptide (CGRP), and prostacyclin synthase (PGIS) has been shown to be effective in model animals (Champion et al. 1999, 2000; Christman et al. 1992; Tuder et al. 1999; Nagaya et al. 2000).

2.6 Cerebral Vascular Disease

Several possible targets for gene therapy in treating cerebral vascular disease have been proposed: (1) prevention of vasospasm after subarachnoid hemorrhage (SAH), (2) protection against brain damage after ischemic stroke, (3) stimulation of collateral vessel formation in areas at risk of ischemia, (4) prevention of restenosis after angioplasty of the carotid and vertebrobasilar arteries, (5) inhibition of thrombosis.

Vasospasm is a serious problem after SAH and, currently, there is no effective method of prevention. Vasospasm is a great potential target of gene therapy because it occurs several days after the occurrence of SAH, so that there is sufficient time to deliver a gene (Toyoda et al. 2003). Moreover, the risk of vasospasm is highest during the 2 to 3 weeks after SAH, a short enough period of time to allow transient gene expression. The mechanisms of vasospasm after SAH may include impaired endothelium-dependent vasorelaxation, production of endothelium-derived contracting factors (endothelin, etc.), and impaired activity of potassium channels in cerebral vessels.

The in vivo transfer of the gene encoding endothelial NOS improved the NO mediated relaxation of the basilar arteries in vitro after experimental SAH (Onoue et al. 1998). Vasospasm in transgenic mice that overexpressed CuZn-SOD or EC-SOD was less severe after experimental SAH (Kamii et al. 1999); McGirt et al. 2002). Vascular contraction was inhibited after SAH by intracisternal administration of preproendothelin-1 antisense oligoDNA, which reduced production of endothelin peptide (Onoda et al. 1996).

CGRP has potent activity in opening potassium channels, hyperpolarizing arterial muscle, and dilating arteries. After SAH, CGRP was shown to depleted from nerves supplying cerebral arteries. The genetic transfer of prepro-CGRP prevented vasospasm in rabbits after experimental SAH (Nozaki et al. 1989; Edvinsson et al. 1991).

The therapeutic targets and genes used for gene therapy of vascular diseases are summarized in Table 1.

TABLE 1. Therapeutic target and genes used for gene therapy in vascular disease

Therapeutic target	Treatment genes
Therapeutic angiogenesis	VEGF-A, -B, -V, -D, -E, FGF-1, -2, -4, -5, angiopoetin-1, HGF, MCP-1, PDGF, eNOS, iNOS, adrenomedullin
Restennosis, vein-graft failure	VEGF-A, C, eNOS, iNOS, COX, Thymidine kinase, CNP Fas ligand, p16, p21, p27, p53, NFkB and E2F decoys, cdk-2, cdc-2, c-myb, c-myc, ras, bcl-x, PCNA antisense oligonucleotides Ribozimes, Bloking PDGF or TGF-β expression or their receptors
Atherosclerosis, hyperlipidaemia	LDL receptor, VLDL receptor, apoA-1, Lipoprotein lipase, Hepatic lipase, LCAT, apoB, Lipid transfer proteins, Lp(a) inhibition, Soluble scavenger-receptor decoy, Soluble VCAM or ICAM, SOD, PAF-AH
Thrombosis	Hirudin, tPA, thrombomodulin, COX, TFPI
Pulmonary hypertension	Prepro-calcitonin gene related peptide, ANP, eNOS, prostacyclin syntase, VEGF-A, adrenomeullin
Vasospasm after SAH	Endothelial NOS, ECSOD, CuZnSOD, Antisense preproendothelin-1, Prepro-CGRP

3 Cancer

Cancer is a genetic disease in which individual cells have mutations in genes related to growth control and apoptosis. In addition, cancer cells also have support systems to promote invasion and metastasis. Tumor growth is a result of the interaction of cancer cells with their microenvironment, including the extracellular matrix, immune system cells and cells involved in angiogenesis; therefore, each of these steps can serve as a target in controlling tumor growth. The strategies for cancer gene therapy can be directed at the tumor itself or at the host. Strategies directed at the tumor involve killing the tumor cells or slowing down their growth by, for example, introducing tumor suppressor genes, suppressing protooncogenes, and inducing prodrug/suicide, or apoptosis. Strategies direct at the host involve the inhibition of angiogenesis, the protection of normal tissues and increasing immunity.

3.1 Tumor Suppressor Genes

In order to target growth regulation in cancer cells, tumor suppressors, that inactivate the growth of some tumors, can be introduced (Bookstein et al. 1993). Since mutations in *p53* are widespread in human cancer, this gene may be the first target for the genetic therapy of cancer using tumor suppressors. The *p53* gene encodes a transcription factor involved in the regulation of the cell cycle and apoptosis. Gene transfer of *p53* to *p53*-defective cells resulted in cessation of cell growth or the induction of apoptosis (Yen et al. 2000; Horio et al. 2000). The transfer of other tumor suppressor genes, such as the retinoblastoma (*Rb*), *p16*, *pTEN* and *mda-7* genes, also effected suppression of tumor growth, (Demers et al. 1998; Jarrard et al. 1997; Lu et al. 1997).

3.2 Protooncogenes

Protooncogenes are activated by overexpression due to gene amplification, point mutations, modification of regulatory elements leading to increased transcription, and rearrangements. The products of these genes include growth factors or their receptors (EGF, EGFR), signal transduction proteins (ras, PI3 kinase), transcription factors (myc, fos, jun) (Isaacs et al. 1995; Konishi et al. 1997; Roylance et al. 1997), and suppressors of apoptosis (bcl-2). Gene therapy strategies to correct protooncogene activation include the transfer of dominant negative gene products, antisense oligodeoxynucleotides, ribozymes, and small interference RNAs. For example, disruption of overexpressed c-*myc* using anti-sense c-*myc* resulted in a 94.5% reduction of tumor size in prostate cancer (Steiner et al. 1998).

3.3 Prodrug/Suicide

The mechanism of prodrug/suicide gene therapy is based on the difference in mitotic activity between normal and cancer cells. The cells are transduced with the herpes simplex virus thymidine kinase (HSV-TK) gene, and are subsequently killed by treatment with the drug acyclovir. The transduced enzyme, HSV-TK, phosphorylates the prodrug into the antiviral compund gancyclovir triphosphate, an inhibitor of DNA

synthesis, which leads to cell death (Ayala et al. 2000; Koeneman et al. 2000; Shalev et al. 2000; Martiniello-Wilks et al. 1998; Hall et al. 1999).

3.4 Apoptosis Induction

The triggering of programmed cell death in tumor cells without affecting normal cells is an attractive therapeutic approach in cancer gene therapy. Ligands that induce apoptosis include tumor necrosis factor (TNF)-α, FasL (Hedlund et al. 1998; Hedlund et al. 1999), and TRAIL (Griffith and Broghammer 2001; Voelkel-Johnson et al. 2002). As TRAIL has the lowest activity in normal tissue, it is an especially promising new therapeutic approach to cancer therapy.

3.5 Inhibition of Angiogenesis

Regulation of the angiogenic switch depends on the local balance between activators and inhibitors. Angiogenesis is triggered by the release of angiogenic stimulators by tumor cells, either as a result of genetic alterations or through activation of the physiologic response to hypoxia, which activates various inducible factors, e.g., transcription factors that trigger the transcription of genes encoding angiogenic stimulators. Many angiogenic factors have already been identified, including VEGF (Ferrara 1999), vascular permeability factor (VPF), FGF, EGF, platelet-derived endothelial growth factor (PD-EGF), PDGF, insulin-like growth factors (IGFs) (Trojan et al. 1994), interleukin-8 (IL-8), transforming growth factor α and β (TGF-α and -β) (Maggard et al. 2001), heparin growth factor, granulocyte colony stimulating factor (GMCSF), E-selectin (Tang et al. 2004), and TNF-α (Claesson-Welsh 2003; Brieger et al. 2003), and used for anti-angiogenic gene therapy of cancers. The goal of this type of therapy is to switch the balance between angiogenic factors and angiogenic inhibitors in the tumor microenvironment to the anti-angiogenic phenotype.

3.6 Increased Immunity

Gene therapy targeted to the immune system has a good likelihood of success as a cancer therapy. In this approach, an antitumor immune response in the host is created, either by immunotherapy, vaccination with cytokine genes, including IL-2 (Belldegrun et al. 2001; Kawakita et al. 1997; Moody et al. 1994; Toloza et al. 1996), IL-4, IL-7, IL-12 (Sanford et al. 2001; Hull et al. 2000; Nasu et al. 1999), GM-CSF (Simons et al. 1999), M-CSF, and interferons.

4 Conclusions

The therapeutic target and genes used for gene therapy in cancer are summarized in Table 2.

TABLE 2. Therapeutic target and genes used for gene therapy in cancer

Aim of Therapeutic Strategy	Target Genes
Induction of tumor suppressor genes	p53, Rb, p16, pTEN, mda-7
Suppression of Protooncogene	EGF, EGFR, ras, PI3 kinase, c-myc, c-fos, jun, Bcl-2
Prodrug/Suiside	HSV-tk (ganciclovir)
Apoptosis Induction	TNFa, Fas ligand
Induction of apoptosis	Bcl-2, c-myc, c-raf, MDM-2, IGF-II, STAT-3 p21, p53, Fas ligand, c-cam, Caspase7 TRAIL, Bax
Inhibition of angiogenesis	Suppression of VEGF, VPF, FGF, EGF, PD-EGF, IGFs IL-9, TGF-a and b, GM-CSF, E-selection, TNF-a
Immunotherapy	IL-2, IL-4, IL-7, IL-12, GM-CSF, Interferons

References

Achen MG, Jeltsch M, Kukk E, Makinen T, Vitali A, Wilks AF, Alitalo K, Stacker SA (1998) Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). Proc Natl Acad Sci USA 95: 548–553

Ayala G, Wheeler TM, Shalev M, Thompson TC, Miles B, Aguilar-Cordova E, Chakraborty S, Kadmon D (2000) Cytopathic effect of in situ gene therapy in prostate cancer. Hum Pathol 31:866–870

Belalcazar LM, Merched A, Carr B, Oka K, Chen KH, Pastore L, Beaudet A, Chan L (2003) Long-term stable expression of human apolipoprotein A-I mediated by helper-dependent adenovirus gene transfer inhibits atherosclerosis progression and remodels atherosclerotic plaques in a mouse model of familial hypercholesterolemia. Circulation 107:2726–2732

Belldegrun A, Tso CL, Zisman A, Naitoh J, Said J, Pantuck AJ, Hinkel A, deKernion J, Figlin R (2001) Interleukin 2 gene therapy for prostate cancer: phase I clinical trial and basic biology. Hum Gene Ther 12:883–892

Benoit P, Emmanuel F, Caillaud JM, Bassinet L, Castro G, Gallix P, Fruchart JC, Branellec D, Denefle P, Duverger N (1999) Somatic gene transfer of human ApoA-I inhibits atherosclerosis progression in mouse models. Circulation 99:105–110

Bookstein R, MacGrogan D, Hilsenbeck SG, Sharkey F, Allred DC (1993) p53 is mutated in a subset of advanced-stage prostate cancers. Cancer Res 53:3369–3373

Brieger J, Bedavanija A, Lehr HA, Maurer J, Mann WJ (2003) Expression of angiogenic growth factors in acoustic neurinoma. Acta Otolaryngol 123:1040–1045

Brousseau ME, Wang J, Demosky SJ, Jr., Vaisman BL, Talley GD, Santamarina-Fojo S, Brewer HB, Jr., Hoeg JM (1998) Correction of hypoalphalipoproteinemia in LDL receptor-deficient rabbits by lecithin:cholesterol acyltransferase. J Lipid Res 39:1558–1567

Burgess TL, Fisher EF, Ross SL, Bready JV, Qian YX, Bayewitch LA, Cohen AM, Herrera CJ, Hu SS, Kramer TB (1995) The antiproliferative activity of c-myb and c-myc antisense oligonucleotides in smooth muscle cells is caused by a nonantisense mechanism. Proc Natl Acad Sci U S A 92:4051–4055

Champion HC, Bivalacqua TJ, D'Souza FM, Ortiz LA, Jeter JR, Toyoda K, Heistad DD, Hyman AL, Kadowitz PJ (1999) Gene transfer of endothelial nitric oxide synthase to the lung of the mouse in vivo. Effect on agonist-induced and flow-mediated vascular responses. Circ Res 84:1422–1432

Champion HC, Bivalacqua TJ, Toyoda K, Heistad DD, Hyman AL, Kadowitz PJ (2000) In vivo gene transfer of prepro-calcitonin gene-related peptide to the lung attenuates

chronic hypoxia-induced pulmonary hypertension in the mouse. Circulation 101: 923–930
Chang MW, Barr E, Lu MM, Barton K, Leiden JM (1995a) Adenovirus-mediated overexpression of the cyclin/cyclin-dependent kinase inhibitor, p21 inhibits vascular smooth muscle cell proliferation and neointima formation in the rat carotid artery model of balloon angioplasty. J Clin Invest 96:2260–2268
Chang MW, Barr E, Seltzer J, Jiang YQ, Nabel GJ, Nabel EG, Parmacek MS, Leiden JM (1995b) Cytostatic gene therapy for vascular proliferative disorders with a constitutively active form of the retinoblastoma gene product. Science 267:518–522
Christman BW, McPherson CD, Newman JH, King GA, Bernard GR, Groves BM, Loyd JE (1992) An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. N Engl J Med 327:70–75
Cioffi L, Sturtz FG, Wittmer S, Barut B, Smith-Gbur J, Moore V, Zupancic T, Gilligan B, Auerbach R, Gomez F, Chauvin F, Antczak M, Platika D, Snodgrass HR (1999) A novel endothelial cell-based gene therapy platform for the in vivo delivery of apolipoprotein E. Gene Ther 6:1153–1159
Claesson-Welsh L (2003) Signal transduction by vascular endothelial growth factor receptors. Biochem Soc Trans 31:20–24
Demers GW, Harris MP, Wen SF, Engler H, Nielsen LL, Maneval DC (1998) A recombinant adenoviral vector expressing full-length human retinoblastoma susceptibility gene inhibits human tumor cell growth. Cancer Gene Ther 5:207–214
Dichek DA, Anderson J, Kelly AB, Hanson SR, Harker LA (1996) Enhanced in vivo antithrombotic effects of endothelial cells expressing recombinant plasminogen activators transduced with retroviral vectors. Circulation 93:301–309
Dvorak HF, Brown LF, Detmar M, Dvorak AM (1995) Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 146:1029–1039
Edvinsson L, Ekman R, Jansen I, McCulloch J, Mortensen A, Uddman R (1991) Reduced levels of calcitonin gene-related peptide-like immunoreactivity in human brain vessels after subarachnoid haemorrhage. Neurosci Lett 121:151–154
Excoffon KJ, Liu G, Miao L, Wilson JE, McManus BM, Semenkovich CF, Coleman T, Benoit P, Duverger N, Branellec D, Denefle P, Hayden MR, Lewis ME (1997) Correction of hypertriglyceridemia and impaired fat tolerance in lipoprotein lipase-deficient mice by adenovirus-mediated expression of human lipoprotein lipase. Arterioscler Thromb Vasc Biol 17:2532–2539
Ferrara N (1999) Molecular and biological properties of vascular endothelial growth factor. J Mol Med 77:527–543
Ferrara N (2000) VEGF: an update on biological and therapeutic aspects. Curr Opin Biotechnol 11:617–624
Ferrara N (2001) Role of vascular endothelial growth factor in regulation of physiological angiogenesis. Am J Physiol Cell Physiol 280:C1358–C1366
Galzie Z, Kinsella AR, Smith JA (1997) Fibroblast growth factors and their receptors. Biochem Cell Biol 75:669–685
Goldstein JL HH, Brown MS (2001) Familial hypercholesterolemia. Vol chap. 120. McGraw-Hill, New York
Gough PJ, Raines EW (2003) Gene therapy of apolipoprotein E-deficient mice using a novel macrophage-specific retroviral vector. Blood 101:485–491
Greeve J, Jona VK, Chowdhury NR, Horwitz MS, Chowdhury JR (1996) Hepatic gene transfer of the catalytic subunit of the apolipoprotein B mRNA editing enzyme results in a reduction of plasma LDL levels in normal and watanabe heritable hyperlipidemic rabbits. J Lipid Res 37:2001–2017
Griffith TS, Broghammer EL (2001) Suppression of tumor growth following intralesional therapy with TRAIL recombinant adenovirus. Mol Ther 4:257–266