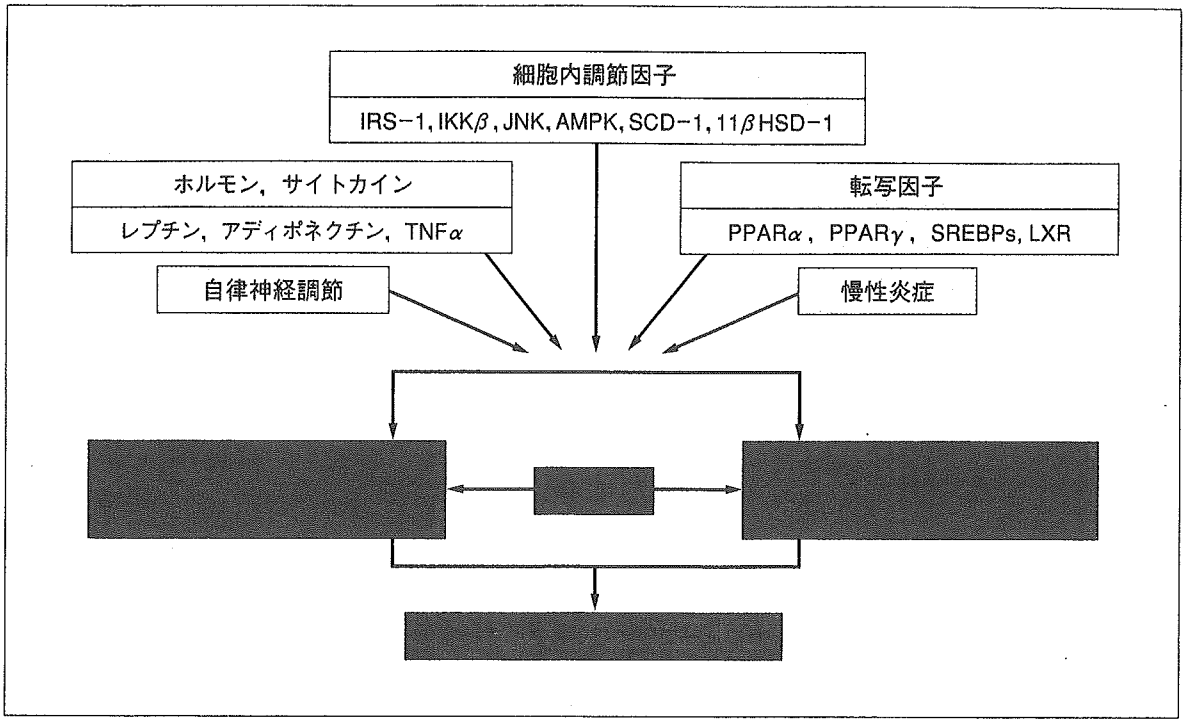
図2　メタボリックシンドロームの原因となる標的因子



略語：巻末の「今月の略語」参照

る因子を同時に解析に加える誤りが，血圧関連因子が単独で抽出されてしまう理由であることが指摘されている．よって，因子分析の結果は慎重に解釈しなければならないが，メタボリックシンドロームが内包する異なった病態がそれぞれに持つ遺伝的背景を明らかにすることが，メタボリックシンドロームのより正確な診断と効果的な臨床介入に重要であることは明白である．

メタボリックシンドロームの候補遺伝子

Reaven 以来，メタボリックシンドロームの成因的基盤をなすものとして「インスリン抵抗性の亢進」が注目されている[7]．例えば，インスリンシグナル伝達機構の1分子を欠損するマウスでは，骨格筋を中心としたインスリン抵抗性の亢進に加え，メタボリックシンドロームに特徴的な脂質代謝異常と高血圧を呈することが報告されている[8]．また，脂肪細胞はインスリン感受性や糖・脂質代謝，血圧の調節にあずかるさまざまな生理活性物質（アディポサイトカイン）を産生しており，脂肪細胞自体の異常もインスリン抵抗性やメタボリックシンドロームの発現を規定・修飾する因子であることが示されている．このようなメタボリックシンドロームの原因となりうる標的因子（図2）に関連する遺伝子は，メタボリックシンドロームの疾患感受性を規定する有力な候補遺伝子である．実際に，多数の候補遺伝子上の変異や多型とメタボリックシンドロームに関連する表現型との間で広く相関研究（association study）が行われ，複数の表現型との間に有意な相関が見られる遺伝子が幾つか報告されている（表1）．これらの中で，PPARγ 遺伝子の Pro12Ala 多型は2型糖尿病の発症リスクを 25% 程度増加するとされ，これは日本人においても確認されている[9]．また，アディポネクチン遺伝子の Ile164Thr 変異が日本人において低アディポネクチン血症と糖尿病に関連し，メタ

表1　メタボリックシンドローム関連遺伝子

遺伝子名	染色体上の局在	多型 / 変異	関連する表現型
LMNA（ラミン A/C）	1q21	H566H	TG, HDL & MS
CAPN10（カルパイン 10）	2q37	SNP−43	BS, TG（肥満者で）
PPARG（PPARγ）	3p25	P12A	TG, HT, IR & MS
Adiponectin（アディポネクチン）	3q27	I164T	MS
GCCR（グルココルチコイド受容体）	5q31	RFLP	Ob, HT, IR
ADRB2（β_2 アドレナリン受容体）	5q32−34	G16R	MS（男性で）
NOS3（内皮依存性 NO 合成酵素）	7q36	7164G−T, D298D	HT & MS
INS（インスリン）	11p15	RFLP	TG & MS
APOC3（アポタンパク質 C3）	11q23	455T−C	TG, Ob & MS
APOC3/A4/A5（アポタンパク質 C3/A4/A5）	11q23	SNP	Ob, HT, IR, TG
INPPL1（SHIP2）	11q23	SNP	HT & MS
GNB3（G タンパク質 β_3 サブユニット）	12p13	C825T	HT & MS
UBL5（BEACON）	19p13	SNP	BS, TG, Ob
PPARA（PPARα）	22q12−13	L162V	TG, HDL, Ob
その他の遺伝子：ADIPOR1（アディポネクチン受容体 1 型），ADRB3（β_3 アドレナリン受容体），LEP（レプチン），LPL（リポタンパク質リパーゼ），PTP1B（タンパク質チロシン脱リン酸化酵素 1B），UCP1 & 2（脱共役タンパク質 1 & 2）			

SNP：単一塩基多型，RFLP：制限酵素切断断片長多型，TG：高トリグリセリド血症，
HDL：低 HDL コレステロール血症，MS：その他のメタボリックシンドロームの表現型，BS：糖代謝異常，
HT：高血圧，IR：インスリン抵抗性，Ob：肥満

ボリックシンドロームおよび冠動脈疾患とも関連する可能性が報告されている[10]．

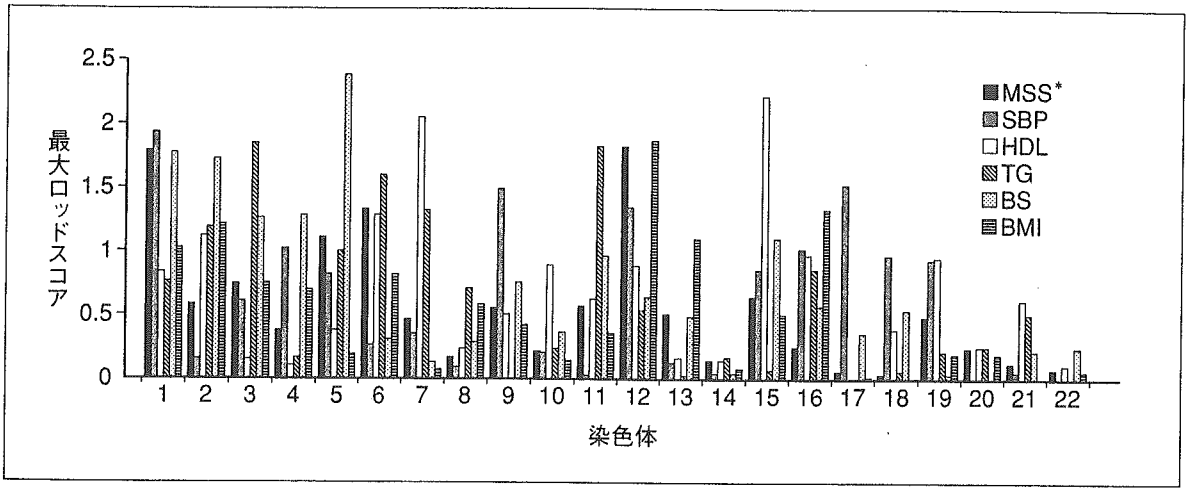
ゲノムワイド連鎖解析による原因遺伝子座の探索

家系内や集団中で複数の遺伝子座が同時に分離（遺伝）されていく現象を連鎖（linkage）と呼び，それは各遺伝子座が同一染色体上の近傍に位置することを意味する．この「連鎖」の概念に基づき，遺伝子マーカーと原因遺伝子との間の組み換え率から原因遺伝子の染色体上の位置を推定する方法が連鎖解析法である．ヒトゲノムの解読により，ゲノム全体に高密度に散在するマイクロサテライトマーカーや単一塩基多型（SNP）などの遺伝子多型のカタログ化が進み，ゲノム全体を俯瞰した連鎖解析や相関解析が可能となりつつある．

1．複数の形質を同時に対象とした解析

Framingham Heart Study の登録者を対象に，メタボリックシンドロームに関連する5つの形質（収縮期血圧，トリグリセリド，HDL コレステロール，血糖値，BMI）およびこれら5つを複合した形質（メタボリックシンドロームスコア：MSS と定義）に対して，マイクロサテライトマーカーを用いた全ゲノム解析を行った結果が最近報告されている（この場合の対象形質はすべて数量化可能なものであるため，量的形質遺伝子座：QTL 解析とも呼ばれる）[11]．その結果，おのおのの形質や MSS に比較的弱い連鎖を示すピークが幾つかの染色体に散在して見られたが，ロッドスコアで 3.3 以上とされる有意（significant）な連鎖は認められず，1.9 以上とされる示唆的（suggestive）な連鎖がわずかに認められた（図3）．各形質および MSS の

図3　メタボリックシンドロームとその形質に関するQTLゲノム解析の結果（文献[11]より引用改変）



* MSSとは，SBP，HDL，TG，BS，BMIの5つの形質に関する標準誤差を総和し，年齢および性別で補正したものと定義．
SBP：収縮期血圧，HDL：HDLコレステロール，TG：トリグリセリド，BS：血糖値，BMI：体格指数

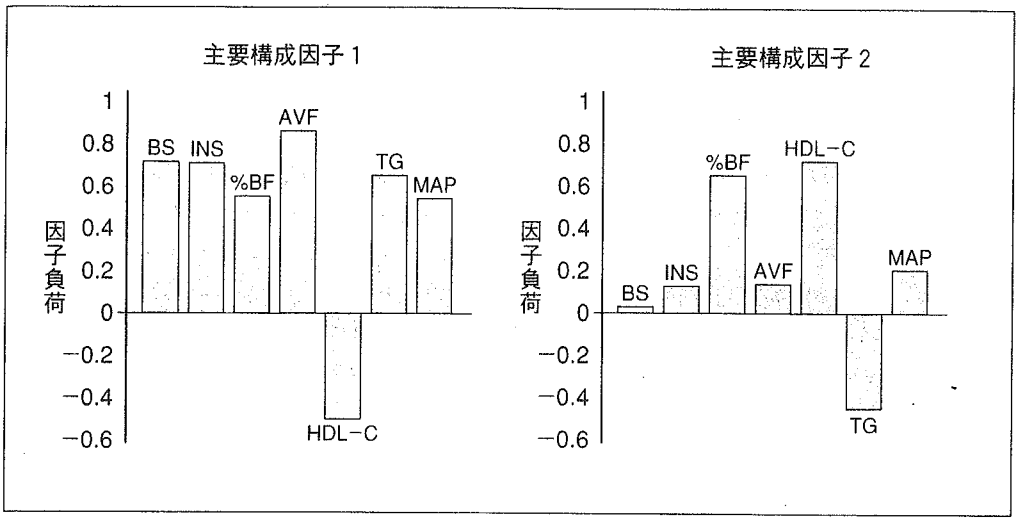
表2　各形質の遺伝度，最大ロッドスコア，QTL領域（Framingham Heart Study）
（文献[11]より引用改変）

形質	遺伝度	ロッドスコア	QTL（領域）	cM**
収縮期血圧	0.48	1.93	1p31	97
HDLコレステロール	0.62	2.27	15p13-q11	1
トリグリセリド	0.56	1.85	3p26	9
血糖値	0.39	2.37	5pter	0
体格指数（BMI）	0.51	1.86	12q21	85
メタボリックシンドロームスコア（MSS）*	0.61	1.82	12q13	63

* MSSは，上記5つの形質に関する標準誤差を総和し，年齢および性別で補正したものと定義．
**染色体の短腕端からの距離，cM：センチモルガン．
略語：巻末の「今月の略語」参照

遺伝度はやはり0.39～0.62と高く，特に5つの形質を合算したMSSの遺伝度が0.61と極めて高いことは，メタボリックシンドロームの成立に遺伝因子が大きく関与していることを示すものである．しかし一方連鎖のピークに関しては，血糖値，HDLコレステロール，収縮期血圧のおのおのの最大ロッドスコアがそれぞれ2.37，2.27，1.93と，示唆的なレベルの連鎖が確認されたのに対して，MSSでは1.82とむしろそれらを下回るロッドスコアしか得られていない（表2）．言い換えるとこの結果は，メタボリックシンドロームの診断基準に用いられる5つの形質に関して，それらの組み合わせや重みづけに関する考慮をせずに単純にその総和をもってメタボリックシンドロームを定義するとすれば，遺伝因子の検出力はおのおのの形質単独の場合に比べて必ずしも改善されないことを示している．これは，「メタボリックシンドロームは複数の独立した構成因子（病態）からなる」という因子分析の結果に合致し，今後メタボリックシンドロームの遺伝解析を行ううえでは，各構成因子に焦点を合わせた解析が重要になるものと思われる．このように

図4　メタボリックシンドロームの主要因子分析の結果（HERITAGE Family Study）
（文献[12]より引用改変）



BS：血糖，INS：インスリン，%BF：体脂肪率，AVF：腹部内臓脂肪，
HDL−C：HDL コレステロール，TG：トリグリセリド，MAP：平均動脈圧

メタボリックシンドロームは複数の独立した構成成分からなるため，何に重点を置くかによって解析対象が異なり，その結果同定される疾患感受性遺伝子も異なり，また解析対象となる人種の違いも結果に大きな影響を及ぼしうることなどに注意が必要である．

2．構成因子を対象とした解析例

メタボリックシンドロームに注目したゲノムワイド連鎖解析は，従来は上記のようにメタボリックシンドロームに関連した複数の形質を同時に対象としたものが主であったが，最近では，因子分析によって抽出された各構成因子を解析対象として，全ゲノムワイド連鎖解析を行ったものも報告されつつある．HERITAGE Family Study における 456 人の白人および 217 人の黒人を対象とした，そのような研究結果が報告されている[12]．まず，因子分析の結果2つの主要な構成因子が抽出され，主要因子1は図4に示すように7つの形質すべてに関連（因子負荷の絶対値＞0.4 が有意）し，全体の分散の 40% を説明する因子であるとされた．また，主要因子2は体脂肪率，HDL コレステロール，トリグリセリドに関連し，全体の分散の 15% を説明する因子であるとされた．

次いで，これらの主要構成因子に関して全ゲノム解析が行われ，幾つかの連鎖を示唆する領域が得られたが，驚いたことに白人と黒人で示された連鎖領域は大きく異なっており，重複する領域は全く認められなかった（表3）．このことはメタボリックシンドロームの成因に人種差が極めて大きく関係することを意味し，我が国におけるメタボリックシンドロームはやはり我が国の独自のデータに基づいて診断および解釈されるべきであることを示している．

3．ゲノムワイド連鎖解析のまとめ

現在までに，多数の疾患感受性遺伝子座位が報告されているが，中でも第7染色体長腕 7q21–31 や第6染色体長腕 6q22–26，および第1染色体長腕 1q21–31 の各領域は複数の研究で報告されており，これらの領域にメタボリックシンドロームの主要な疾患感受性遺伝子が存在する可能性が高い[13]．特に，

表3　メタボリックシンドロームの主要構成因子を対象とした全ゲノム解析の結果（HERITAGE Family Study）（文献[12]より引用改変）

	染色体領域	短腕端からの距離（cM）	構成因子	Multipoint P-value
白人	1q41	233	因子1	0.0097
	2p22	45～48	因子2	0.0045
	9q13	40	因子1	0.0095
	10p11	27～38	因子1	0.0003
	19q13	59	因子2	0.0009
黒人	1p34	54～56	因子2	0.0011
	7q31	134	因子2	0.0071
	9q21	60	因子2	0.0049

HERITAGE Family Study で黒人におけるメタボリックシンドロームの主要な原因遺伝子座が存在する可能性が示された第7染色体の長腕領域は，ヒスパニックを対象とした同様の研究においても強い連鎖を示し[14]，大いに注目される．

おわりに

メタボリックシンドロームに関する因子分析の結果を中心として，メタボリックシンドロームの疾患感受性を規定する因子と幾つかの候補遺伝子，そしてゲノムワイド連鎖解析の結果に関して述べた．因子分析の結果に示されるように，メタボリックシンドロームはおそらくは複数の異なった病態を内包する概念であり，将来的にはおのおのの主たる成因に従った細分化が必要になるものと思われる．メタボリックシンドロームの遺伝的背景を明らかにすることはその端緒となり，またメタボリックシンドロームのより正確な診断と効果的な臨床介入に重要となろう．

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Genetic Susceptibility to the Metabolic Syndrome

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Gene Transfer and Target Diseases

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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

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