FHヘテロ接合体のリスクの診断とリスクカテゴリー分類

FHヘテロ接合体患者の臨床症状は，症例によって動脈硬化の発症年齢や進展速度に大きな幅があることが知られている。FHヘテロ接合体の動脈硬化発症，進展を決定している主要リスク因子として，年齢，性別，アキレス腱肥厚，LDL受容体遺伝子変異の部位，HDLコレステロール値などが報告されている。これらの主要リスク因子を**表3**に示す。FHヘテロ接合体において，所有する主要リスクの数(**表4**)に応じてFH追加リスク0～1群，FH追加リスク2以上群に分類できる。

FHの動脈硬化の診断

FH患者は診断時に一度は専門医を受診して動脈硬化症の診断も行うこと，さらに，半年～1年ごとに専門医でフォローし，動脈硬化性疾患の早期診断，早期治療に努めるべきである。FHヘテロ接合体は1～2年ごとにCADの診断を行う。また，この他には，ankle-brachial blood pressure index (ABI)，頸動脈エコー，腹部エコーを行い，大腿動脈，頸動脈の動脈硬化および腹部大動脈瘤の評価を行う。

FHの治療

FHの治療の基本は，CADなど若年齢で起きる動脈硬化症の発症および進展の予防であり，早期診断と適切な治療が最も重要である。FHはできるだけ早期に診断を下し，低脂肪食などの正しい食生活を子供時代から身につけると同時に，喫煙，肥満などの動脈硬化症の増悪因子をしっかりと避け，高血圧や糖尿病を厳格にコントロールする。しかしながら，生活習慣の改善のみでは，LDLコレステロール値を安全域まで十分に低下させることは困難であり，以下に記述する薬物療法を必要とする。

(1)FHヘテロ接合体患者のコントロール目標

FHヘテロ接合体のLDLコレステロール目標値は，患者が有する主要リスクに応じて設定する(**表4**)。すなわち，FH追加リスク0～1群は120mg/dL，FH追加リスク2以上群は100mg/dLとする。

(2)FHヘテロ接合体患者の薬物療法

FHヘテロ接合体患者に対する薬物療法については，男性はコレステロール合成経路の律速酵素であるHMG-CoA還元酵素阻害薬(スタチン)が第一選択である。FHヘテロ接合体に対しては，LDLコレステロール値の低下効率から考えると，ストロングスタチンが第一選択薬になる場合が多い。スタチンは初期用量から増量し，LDLコレステロール値の低下効果は用量依存的であるが，副作用の頻度と重症度も増すことがある。スタチンに加えて，他

の薬効を有する薬剤を併用すると，よりLDLコレステロールの低下効果が得られることが報告されている。スタチン単剤で十分な効果が得られない場合，コレステロール吸収阻害薬であるエゼチミブ，胆汁酸吸着レジンであるコレスチラミンやコレスチミド，あるいはプロブコールなどが併用されている。女性の場合はスタチンは妊娠時には絶対禁忌であるため妊娠の可能性のある場合はレジンによる治療を考慮する。リスクに応じて薬物治療を考慮する。

薬物治療開始後，3カ月間は毎月，問診で筋痛などの筋肉の症状の有無を問い，LDLコレステロール，HDLコレステロール，トリグリセリドを測定して効果の判定を行うと同時に，AST，ALTなどの肝機能をはじめCPKを測定して，副作用の発現に注意する。3カ月後からは，3カ月に1回は上記の検査を行い，副作用のなかでも最も重篤な横紋筋融解症を見逃さないように注意する。

(3)FHヘテロ接合体患者のLDLアフェレシス療法

薬物を使用しても血清総コレステロール値が250mg/dL以下に低下せず，明らかな冠動脈硬化を有する場合，体外循環により血漿LDLを直接取り除くLDLアフェレシスの適応となる(健康保険が適用される)。日本人において，CADを有するFHヘテロ接合体に対するLDLアフェレシスの有効性を証明するデータが複数報告されている。強力なLDLコレステロール値低下作用をもつストロングスタチンが市販されてから，これらのスタチンとの併用で，より厳密なLDLコレステロールの管理が可能になった。

表3 FHヘテロ接合体の主要なリスク因子

1. 年齢
   男性
   女性≧45歳または閉経後
2. 喫煙：現在の喫煙
3. 冠動脈疾患の家族歴
4. 未治療時のLDLコレステロール≧260mg/dLあるいはアキレス腱厚(≧15mm)
5. HDLコレステロール<40mg/dLまたはトリグリセリド≧150mg/dL
6. 糖尿病(耐糖能異常を含む)
7. 高血圧

表4 カテゴリーリスクに応じた目標LDL-C値

カテゴリー	主要リスクの数	目標LDL-C値(mg/dL)
FH追加リスク0～1	0～1	120あるいは50%以上の低下
FH追加リスク2以上	2つ以上 あるいは冠動脈，頸動脈などに 明らかな動脈硬化による狭窄を認める	100あるいは50%以上の低下

目標LDL-C値は，実際に到達できるかは現実的に難しい場合がある。その場合でも未治療時のLDL-C値の50%以上の低下を目指す。
また，実際に到達していても，イベントをなくすことを保証するものではない。

(4)FHホモ接合体患者の治療

●FHホモ接合体の薬物療法

胆汁酸吸着レジンやスタチンなど，LDL低下薬の薬効の主要な部分はLDL受容体の活性の増加によるものであり，FHホモ接合体は，FHヘテロ接合体に比べて薬剤に対する反応性が非常に悪い。そのため，1～2週間に1回のLDLアフェレシス治療が必要である。プロブコールは，FHホモ接合体に対しても一定の総コレステロール値の低下効果があり，またそれ以上に皮膚黄色腫の縮小，消失を認める報告がある。

●FHホモ接合体のLDLアフェレシス療法

FHホモ接合体はLDLアフェレシスの絶対適応であり，できるかぎり早期にLDLアフェレシス治療を開始すべきである。現実的な治療開始の時期は，ベッド上で臥床し体外循環施行が可能となる4～6歳ごろからとなる。乳児期にすでに冠動脈狭窄や完全閉塞，大動脈弁狭窄や弁上狭窄を有する例も存在し，開始の時期が遅くなるほど予後が悪くなるので，できるかぎり早期に治療を開始することが勧められる[11]。

特定疾患認定について

家族性高コレステロール血症ホモ接合体が特定疾患として医療費補助の対象になることが決定し，平成21年10月1日より施行された。認定基準は，LDL代謝経路にかかわる遺伝子の遺伝子解析，あるいはLDL受容体活性測定にて確定診断が下される確実例に加えて，ほぼ確実例として著明な高コレステロール血症，あるいは小児期よりの皮膚黄色腫の存在や薬剤治療に抵抗する患者が認定の対象となっている。

おわりに

FHは，早期に正しく診断を下すこと，早期に適切な治療を開始することにより，著明に予後を改善することができる疾患である。動脈硬化の進展の程度を把握してLDLコレステロール値をコントロールするとともに，LDL以外の動脈硬化危険因子である糖尿病，高血圧症，喫煙などの有無をスクリーニングし，厳格にコントロールする必要がある。

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家族性高コレステロール血症をどう扱うか

斯波 真理子

ポイント

★FHは，LDL受容体およびその関連遺伝子の変異による遺伝病であり，常染色体性優性遺伝型式をとる．

★FHの診断には，高LDL-C血症，皮膚結節性黄色腫，腱黄色腫の存在とともに，家系内にFHや若年性冠動脈疾患患者がいることが決め手となる．

★FHは若年性冠動脈疾患を併発するため，食事療法などの生活習慣の改善とともに，薬物療法が必要である．

★FHホモ接合体は極端な高LDL-C血症を示し，薬物に対して抵抗性を示すため，LDLアフェレーシス治療が必要である．

家族性高コレステロール血症(familial hypercholesterolemia：FH)は，高コレステロール血症，皮膚および腱黄色腫，若年性動脈硬化症による冠動脈疾患(CAD)などを主徴とする遺伝性疾患であり，常染色体優性遺伝形式をとる[1]．原因遺伝子としては古くからLDL受容体遺伝子が知られているが，近年，LDL受容体の機能にかかわるproprotein convertase subtilisin/kexin-type 9(PCSK9)やautosomal recessive hypercholesterolemia(ARH)などの遺伝子の報告もなされている[2,3]．FHヘテロ接合体患者は500人に1人以上，ホモ接合体患者は100万人に1人以上の頻度で認められると言われており，わが国におけるFH患者総数は，25万人以上と推定される．最近，日本においてFHの数がさらに多く，ヘテロ接合体で200人に1人，ホモ接合体で17万人に1人であるとの報告もある[4]．FHは遺伝性代謝疾患の中でも最も高頻度であり，日常診療においても高頻度で遭遇する．CAD，大動脈弁狭窄など動脈硬化による血管疾患を高率に引き起こすため，FHの診療においては早期診断および早期治療による動脈硬化症の発症，進展の予防が重要である．

本稿では，FHの診断法，遺伝子解析，治療法について，これまで得られた知見をもとに解説する．

FHの臨床像

血清脂質値

FHヘテロ接合体の臨床所見で最初に現れるのは，高LDL(低比重リポ蛋白)コレステロール血症である．多くの例において出生時より明らかな高LDLコレステロール血症が認められるが[5]，これが唯一の臨床症状である[6]．血清総コレステロール値は，230～500 mg/dl(LDLコレステロール値は150～420 mg/dl)であり，いずれの場合も個々の症例による血漿コレステ

しば まりこ：国立循環器病研究センター研究所分子薬理部 〒565-8565 大阪府吹田市藤白台5-7-1

0025-7699/11/¥500/論文/JCOPY

【表 1】FH Index 法による FH の診断

1. 未治療時の LDL コレステロール値 160～179 mg/dl ……**1 点** 180～199 mg/dl ……**2 点** 200 mg/dl 以上 ……**4 点**
2. 家族歴(二親等) 以下の項目に該当の場合……**2 点** ・若年性冠動脈疾患(男性＜55 歳，女性＜65 歳) ・LDL コレステロール値＞180 mg/dl(＞15 歳) または，FH と確定診断されている場合……**4 点**
3. 以下の項目に該当の場合……**6 点** ・腱黄色腫または，皮膚結節性黄色腫が存在する． ・X 線軟線撮影またはゼロラジオグラフィによるアキレス腱肥厚の判定(側面で最大径 9 mm 以上)
4. 若年性角膜輪(＜50 歳)あるいは若年性冠動脈疾患(男性＜55 歳，女性＜65 歳)がある場合……**4 点**
5. LDL レセプター関連遺伝子変異が認められた場合……**8 点** ＊LDL レセプター活性低下(健常人の 80%未満)は診断の参考
各項目ごとの合計点数が， **6 点以上で FH 疑い，** **8 点以上で確定診断とする．**

【表 2】簡易法による FH の診断

1. 高 LDL コレステロール血症 未治療時の LDL コレステロール値≧180 mg/dl
2. 高 LDL コレステロールの身体症状 皮膚結節性黄色腫の存在，あるいは 腱黄色腫(アキレス腱厚さ≧9 mm)
3. 家族歴(二親等以内) FH あるいは若年性冠動脈疾患の家族歴

2 項目あてはまれば FH と確定診断，1 項目あてはまれば，家族内検査，3 カ月後のフォローアップなどを行う．

ロール値のばらつきは大きい．

FH ホモ接合体の血清総コレステロール値は 450～1,200 mg/dl(LDL コレステロール値は 370～900 mg/dl)であり，乳児期より著明高値をとることが多い．

角膜輪と黄色腫

FH ヘテロ接合体の角膜輪や腱黄色腫は 10 歳台後半から現れ，30 歳までに半分の症例に現れる．死亡するまでには，80%の症例でこれらの症状が出現する[7]．角膜輪は，初期には半月状に見えることもあり，瞼を挙上して観察する必要がある．腱黄色腫はアキレス腱のものが最もよく知られており，診断に用いられるが，手背伸筋腱にも発生する[8]．視診のみでも診断できることがあるが，触診が最も重要であり，正常と比較して硬く，肥厚したアキレス腱が触診される．X 線軟線撮影により，9 mm 以上を異常とする[9]．眼瞼黄色腫は FH に特異的なものではなく，正脂血症の患者にも認められる．

FH ホモ接合体は，出生時より著明な高 LDL コレステロール血症を呈し，皮膚黄色腫が特徴的である．

動脈硬化

FH ヘテロ接合体において，冠動脈疾患発症のリスク解析では，男性，加齢，喫煙，高血圧，糖尿病，高 LDL コレステロール血症，低 HDL(高比重リポ蛋白)血症，BMI，CAD の家族歴を有することなどがあることが報告されている[10]．その他のリスク因子としては，高トリグリセリド血症，高 Lp(a)血症，高ホモシステイン血症などの報告がなされている．若年性動脈硬化は大動脈には腹部大動脈瘤として現れることがあり，その頻度は約 26%と報告されている．脳血管疾患については，馬渕らは FH の死亡例 41 例の中での脳卒中死亡率が，一般日本人のものと違いがないことを報告している．閉塞性動脈硬化症は，8～16%の FH 例に合併する．

FH ヘテロ接合体の診断

FH ヘテロ接合体の診断は，未治療時の LDL コレステロール値が高値であること，高 LDL 血症に伴う身体症状である腱黄色腫や角膜輪の存在，若年性動脈硬化症の症状である若年性 CAD(発症年齢:男性 55 歳未満，女性 65 歳未満)

【表3】FHヘテロ接合体の主要なリスク因子

1. 年齢
   男性
   女性：≧45歳または閉経後
2. 喫煙：現在の喫煙
3. 冠動脈疾患の家族歴
4. 未治療時のLDLコレステロール≧270 mg/dl
   あるいはアキレス腱厚(≧15 mm)
5. HDLコレステロール：<40 mg/dl
   またはトリグリセライド：≧150 mg/dl
6. 糖尿病(耐糖能異常を含む)
7. 高血圧

【表4】カテゴリーリスクに応じた目標LDL-C値

カテゴリー	主要リスクの数	目標LDL-C値 (mg/dl)
FH高リスク相当	0～1	120
FH二次予防リスク相当	2つ以上	
	あるいは冠動脈，頸動脈などに明らかな動脈硬化による狭窄を認める	100

目標LDL-C値は，実際に到達できるかは現実的に難しい場合がある．また，実際に到達していても，イベントをなくすことを保障するものではない．

の存在，FHの家族歴(二親等以内)などが診断の根拠となる．FH Index法による診断基準(案)を**表1**に，簡易法によるFH診断基準を**表2**に記す．

FHヘテロ接合体のリスクの診断とリスクカテゴリー分類

FHヘテロ接合体患者の臨床症状は，症例によって動脈硬化の発症年齢や進展速度に大きな幅があることが知られている．FHヘテロ接合体の動脈硬化発症，進展を決定している主要リスク因子として，年齢，性別，アキレス腱肥厚，LDL受容体遺伝子変異の部位，HDL-コレステロール値などが報告されている．これらの主要リスク因子を**表3**に示す．FHヘテロ接合体において，所有する主要リスクの数(**表3**)に応じてFH高リスク相当群，FH二次予防相当リスク群に分類できる．

FHの動脈硬化の診断

FH患者は診断時に一度は専門医を受診して動脈硬化症の診断も行うこと，さらに，半年～1年ごとに専門医でフォローし，動脈硬化性疾患の早期診断，早期治療に努めるべきである．FHヘテロ接合体は1～2年ごとにCADの診断を行う．また，このほかには，ankle-brachial blood pressure index(ABI)，頸動脈エコー，腹部エコーを行い，大腿動脈，頸動脈の動脈硬化および腹部大動脈瘤の評価を行う．

FHの治療

FHの治療の基本は，CADなど若年齢で起きる動脈硬化症の発症および進展の予防であり，早期診断と適切な治療が最も重要である．FHはできるだけ早期に診断を下し，低脂肪食などの正しい食生活を子供時代から身につけると同時に，喫煙，肥満などの動脈硬化症の増悪因子をしっかりと避け，高血圧や糖尿病を厳格にコントロールする．しかしながら，生活習慣の改善のみでは，LDLコレステロール値を安全域まで十分に低下させることは困難であり，以下に記述する薬物療法を必要とする．

FHヘテロ接合体患者のコントロール目標

FHヘテロ接合体のLDLコレステロール目標値は，患者が有する主要リスクに応じて設定する(**表4**)．すなわち，FH高リスク相当群は120 mg/dl，FH二次予防相当リスク群は100 mg/dlとする．

FHヘテロ接合体患者の薬物療法

FHヘテロ接合体患者に対する薬物療法については，コレステロール合成経路の律速酵素であるHMG-CoA還元酵素阻害薬(スタチン)が第一選択である．FHヘテロ接合体に対しては，LDLコレステロール値の低下効率から考えると，ストロングスタチンが第一選択薬になる場合が多い．スタチンは初期用量から増量し，LDLコレステロール値の低下効果は用量依存的であるが，副作用の頻度と重症度も増すことがある．スタチンに加えて，ほかの薬効を有する薬剤を併用すると，よりLDLコレステロールの低下効果が得られることが報告されている．スタチン単剤で充分な効果が得られない場合，コレステロール吸収阻害剤であるエゼチミブ，胆汁酸吸着レジンであるコレスチラミンやコレスチミド，あるいはプロブコールなどが併用されている．

薬物治療開始後，3カ月間は毎月，問診で筋痛などの筋肉の症状の有無を問い，LDLコレステロール，HDLコレステロール，トリグリセライドを測定して効果の判定を行うと同時に，AST，ALTなどの肝機能をはじめCPKを測定して，副作用の発現に注意する．3カ月後からは，3カ月に1回は上記の検査を行い，副作用の中でも最も重篤な横紋筋融解症を見逃さないように注意する．

FHヘテロ接合体患者のLDLアフェレーシス療法

薬物を使用しても血清総コレステロール値が250 mg/dl以下に低下せず，明らかな冠動脈硬化を有する場合，体外循環により血漿LDLを直接取り除くLDLアフェレーシスの適応となる(健康保険が適用される)．日本人において，CADを有するFHヘテロ接合体に対するLDLアフェレーシスの有効性を証明するデータが複数報告されている．強力なLDLコレステロール値低下作用を持つストロングスタチンが市販されてから，これらのスタチンとの併用で，より厳密なLDLコレステロールの管理が可能になった．

FHホモ接合体患者の治療

FHホモ接合体の薬物療法

胆汁酸吸着レジンやスタチンなど，LDL低下薬の薬効の主要な部分はLDL受容体の活性の増加によるものであり，FHホモ接合体は，FHヘテロ接合体に比べて薬剤に対する反応性が非常に悪い．そのため，1〜2週間に1回のLDLアフェレーシス治療が必要である．プロブコールは，FHホモ接合体に対しても一定の総コレステロール値の低下効果があり，またそれ以上に皮膚黄色腫の縮小，消失を認める報告がある．

FHホモ接合体のLDLアフェレーシス療法

FHホモ接合体はLDLアフェレーシスの絶対適応であり，できる限り早期にLDLアフェレーシス治療を開始すべきである．現実的な治療開始の時期は，ベッド上で臥床し体外循環施行が可能となる4〜6歳頃からとなる．乳児期にすでに冠動脈狭窄や完全閉塞，大動脈弁狭窄や弁上狭窄を有する例も存在し，開始の時期が遅くなるほど予後が悪くなるので，できる限り早期に治療を開始することが勧められる[11]．

特定疾患認定について

家族性高コレステロール血症ホモ接合体が特定疾患として医療費補助の対象になることが決定し，2009(平成21)年10月1日より施行され

た．認定基準は，LDL代謝経路にかかわる遺伝子の遺伝子解析，あるいはLDL受容体活性測定にて確定診断が下される確実例に加えて，ほぼ確実例として著明な高コレステロール血症，あるいは小児期よりの皮膚黄色腫の存在や薬剤治療に抵抗する患者が認定の対象となっている．

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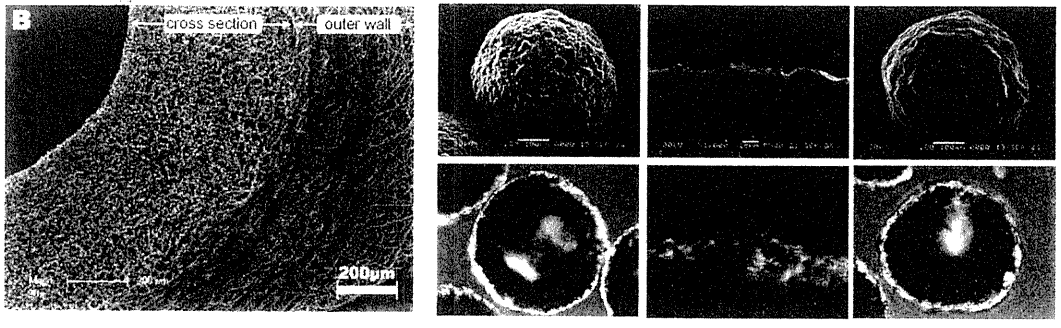
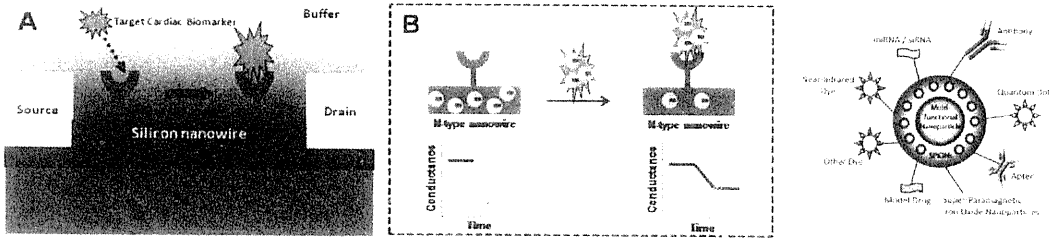
Nanomedicine and the Cardiovascular System





Editors
Ross J. Hunter
Victor R. Preedy

Nanomedicine and the Cardiovascular System

Editors
Ross J. Hunter
Cardiology Research Fellow
St Bartholomew's Hospital
London
UK

Victor R. Preedy
Professor of Nutritional Biochemistry
School of Biomedical & Health Sciences
King's College London
and
Professor of Clinical Biochemistry
King's College Hospital
UK

CRC Press
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Intratracheal Gene Transfer Using Polyplex Nanomicelles and Their Application to Cardiology

Noriyuki Iwamoto[1] and *Mariko Hrada-Shiba*[2,*]

ABSTRACT

Advances in nanotechnology have led to its application in the field of biomaterials, including drug delivery systems (DDS). To carry nucleic acid drugs, other drugs, and genes to the targeted organs, devices that can carry them intact all the way from the site of administration to the target organs are needed. The development of DDS materials is a challenging and attractive research area whose goal is to treat intractable diseases such as metabolic, cardiovascular, and cancer diseases including genetic diseases with least adverse effects.

Nanocarrier systems based on non-viral DDS, such as lipoplexes and polyplexes, including our polymeric nanomicelles from poly(ethylene glycol)-poly(amino acid) block copolymer, are among the most functional devices for gene delivery. To deliver genes successfully to target tissues, three primary factors must be clearly addressed: the gene carrier, the therapeutic gene, and the route of administration. The intratracheal administration of therapeutic genes by using polyplex or lipoplex is a promising strategy for delivering genes because it has many advantages over intravenous administration, which has problems such as the degradation of the gene by the nuclease, binding and aggregation with other proteins or erythrocytes in the blood, and difficulty breaking through endothelial barriers. We recently reported that in polyplex nanomicelles consisting of poly(ethylene glycol)-poly(amino acid) block copolymer and adrenomedullin, a therapeutic gene, could attenuate animal models of pulmonary arterial hypertension. This chapter focuses on challenges in the development of gene carriers, therapeutic genes currently used, and the characteristics of the administration route of successful gene therapy for cardiovascular disease. We also discuss our recent report of intratracheal administration of the adrenomedullin gene by using our delivery systems.

[1]Division of Endocrinology and Metabolism, National Cerebral and Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan; E-mail: niwamoto@hsp.ncvc.go.jp

[2]Department of Molecular Innovation in Lipidology, National Cerebral and Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan; E-mail: mshiba@ri.ncvc.go.jp

*Corresponding author

List of abbreviations after the text.

INTRODUCTION

Gene therapy is one of the strategic approaches to continuously supplying therapeutic peptides or proteins to target tissues. Gene therapy using viral vectors is reported to deliver therapeutic genes effectively, gain access to host cells, and exploit the cellular machinery to facilitate their own replication. However, viral vectors proved to have adverse effects of toxicity, immunogenicity, inflammatory properties, and high cost (Thomas et al. 2003). In addition, an overwhelming immune reaction against adenovirus occurred in a patient at Pennsylvania University in 1999 (Marshall 2000), and a leukemia-like disease was reported in a French patient in 2002 (Marshall 2002).

Non-viral vectors show certain advantages over viral ones in terms of safety, immunogenicity, and ease of manufacture. To obtain successful gene delivery to the target tissue, three primary factors must be clearly addressed: (1) the development of an appropriate gene carrier, (2) the selection of a therapeutic gene, and (3) the selection of an administration route. (1) The development of advanced carrier systems is essential for protecting therapeutic plasmids or drugs in the environment, for providing

site-specific targeting, and for releasing effectively the plasmids or drugs for the desired pharmacological effect. (2) After the genome project, many genes related to the pathogenesis and pathophysiology of diseases were identified and their functions clarified. The appropriate genes that show therapeutic effects in the target tissue should be selected. (3) Selection of a route depending on the target tissue and the gene carrier is essential for successful delivery. Intravenous gene delivery is extremely difficult because of the propensity for rapid nuclease degradation in the blood.

Non-viral delivery systems used to present low levels of transfection and expression efficiency of the gene. Recent advances in the technology of non-viral vectors have yielded techniques with transfection efficiencies similar to those of viruses (Bae and Kataoka 2009). One of the intelligent non-viral gene delivery systems (Fig. 1) is poly(ethylene glycol)(PEG)-based block catiomers, polyplex nanomicelles, composed of a hydrophilic shell and a hydrophobic core. These are well dispersed even in aqueous media containing serum proteins, and they protect plasmid DNA from degradation by nuclease *in vitro* and *in vivo* (Katayose and Kataoka 1998; Wakebayashi et al. 2004; Harada-Shiba et al. 2002). This chapter focuses on the characteristics of non-viral vectors including lipoplexes, polyplexes, and nanomicelle carriers and their application to cardiovascular disease.

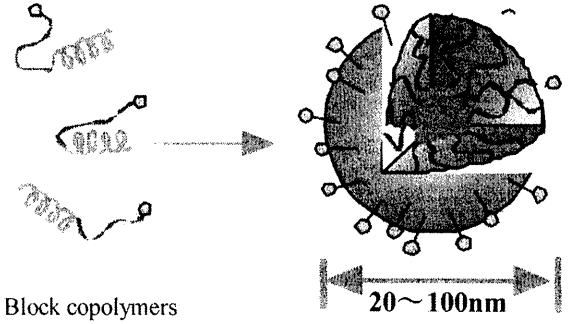

Fig. 1. The schema of the structure of polymeric nanomicelle. Polymeric nanomicelle consists of DNA and block copolymer showing a core shell structure.

Color image of this figure appears in the color plate section at the end of the book.

DEVELOPMENT OF GENE CARRIERS

To achieve safe and effective gene therapy by using a non-viral vector, the construction of a reliable carrier with minimal toxicity is essential. For successful gene delivery to the target cells, the carrier should offer

the following: (1) protection of plasmids from the environment during the route to the target cells, (2) incorporation into the target cells, (3) endosomal escape, and (4) transportation to the nucleus.

From Administered Site to Target Cells

The first step of the targeting process is the compartmentalization of plasmid DNA into a carrier that is resistant to the circumstances during the route to the target cells. This step includes compaction or condensation of DNA using polycations like chromatin structure, which is condensed by the electrostatic attractions between the lysine residues around the histone octamer and DNA. This compaction and condensation step is useful for the polyplex to become a nanoparticle that is not recognized by the immune system and not digested by nuclease.

The second step in this process is the internalization of DNA into the target cells. The positively charged DNA complexes are known to interact with anionic substances on the cell surface, such as proteoglycan and sialic acid, by electrostatic interaction. The internalization process of the polyplexes may be affected by their size and zeta potential. Administration of positively charged polyplexes into the blood gives aggregation with negatively charged proteins that are abundantly reserved in the blood and rapidly removed by reticulo-endothelial systems. On the other hand, the polyplexes in neutralized condition form secondary aggregates, which may cause the formation of large precipitates. The polyplex system consisting of cationic homopolymer and DNA is appropriate for examining the functional properties of complexation and condensation of DNA. However, it is not suitable for intravenous administration.

Cationic block copolymer, consisting of hydrophilic PEG and cationic polymer, produces DNA condensate through the formation of polyion complex (PIC) micelles that are surrounded by a hydrophilic layer (Katayose 1998) presenting a nano-sized structure, as shown in the electron microscopic image (Fig. 2) (Harada-Shiba 2002). The formation of micelles with cationic block copolymer and DNA gives colloidal stability in a protein-containing medium, a favorable property for *in vivo* use.

Receptor-mediated Gene Delivery

Wu et al. developed a system for delivering genes to hepatocytes via a unique receptor that can bind and internalize the galactose terminal of asialoglycoprotein (Wu et al. 1987). It was shown that asialoorosomucoid-poly-L lysine carriers delivered DNA specifically to hepatoma cells (Wu et al. 1988a, b). The cell-specific delivery received much attention, and ligands including lactose, folate, transferrin, oligopeptides with arginin-glycine-aspartic acid (RGD), low-density lipoprotein (LDL), antibody, and

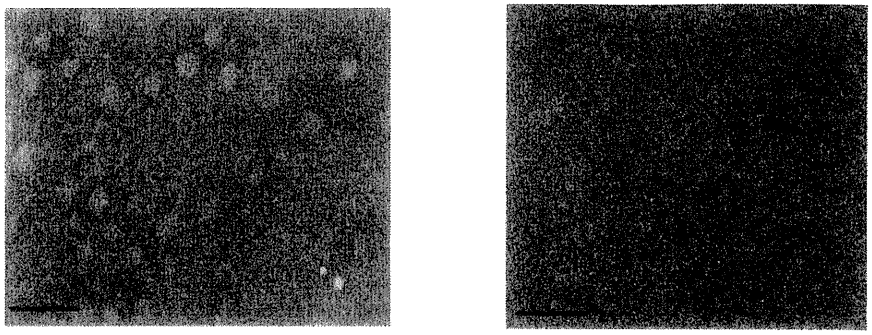

Fig. 2. Electron microscopic findings of negatively stained polyion complex (PIC) nanomicelles. The nanomicelles were prepared by mixing pGL3-control plasmid and PEG-poly-L-lysine block copolymer at the charge ratio of 1:2 (a) and 1:4 (b). Bar equals 100 nm. The data are taken from Harada-Shiba (2003).

so on, were tried targeting hepatocytes, hematopoietic stem cells, epithelial cells, and other cells (Table 1). The polyethyleneimine (PEI) derivatives conjugated to the integrin-binding peptide CYG-GRGDTP via a disulfide bridge led to transgene expression in integrin-expressing epithelial cells (Hela) and fibroblasts at expression levels 10 to 100 times those obtained by PEI alone. The advantage of receptor-mediated gene delivery is not only the specificity of the cells but also the controlled intracellular trafficking of the delivered complex (Erbacher et al. 1999).

Receptor-mediated gene delivery has also been developed using cationic liposomes, whose transfection efficiency is higher than that of cationic polymers. Effective hepatocyte targeting was reported by using galactosylated cationic liposomes. However, because the introduction of asialoglycoproteins to liposomes appeared to have some problems in reproducibility and immunogenicity, low-molecular-weight glycolipids were found to be more promising by virtue of their low immunogenicity and high reproducibility. Intravenously administered DNA/cationic liposome

Table 1. Receptors and ligands targeted for gene transfer.

Receptor	Ligand	Target cells
Asialoglycoprotein receptor	Asialoorosomucoid Glycosylated polylysine	Hepatocytes
LDL receptor	LDL	Hepatocytes
Folate receptor	Folate	Ubiquitous
Epidermal growth (EGF) receptor	Anti-EGF antibody	Ubiquitous
Integrins	RGD	Ubiquitous
Mannose receptor	Glycosylated polylysine	Macrophage
Transferrin receptor	Transferrin	Ubiquitous

complexes are reported to interact with erythrocytes, which suggests that DNA/cationic liposome complexes are aggregated by non-specific interaction with erythrocytes. The modification of cationic liposomes with PEG lipids was reported to reduce the aggregation (Eliyahu et al. 2002).

A System that Can Enable the Endosomal Escape

When DNA is incorporated into the target cell via a non-viral vector—whether by non-specific endocytosis, phagocytosis, pinocytosis, or receptor-mediated endocytosis—it is separated from the cytoplasm by the vesicle membrane, called endosome. The internal pH of endosomes containing DNA complexes gradually decreased to about 5.5 to become late endosomes, after which the endosomes fuse with lysosomes to become secondary lysosomes where the incorporated DNA complexes are usually hydrolyzed by lysosomal enzymes. This step is recognized as the biggest barrier to successful gene delivery and expression (Fig. 3). In order to let the transferred gene express, the gene must escape from the endosomal vesicles and reach the nucleus. When naked DNA is introduced into the cytosol of the cell by microinjection or osmotic shock, much higher expression of the gene is obtained than in the coculture method. This suggests that lysosomal digestion is the key to getting better transfection efficiency.

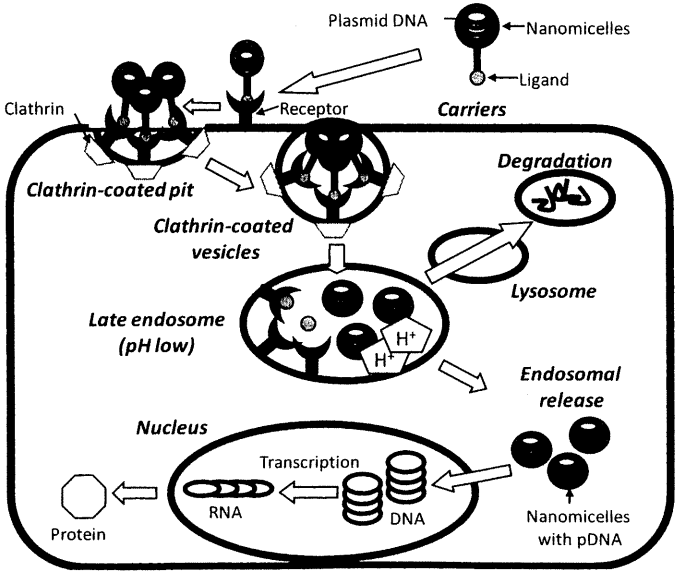

Fig. 3. The schema of intracellular trafficking of non-viral gene delivery systems. The route of nanomicelles from the binding to the cell surface, incorporation, endosomal release, nuclear targeting, transcription, and translation is shown.

Much attention has been paid to promote endosomal escape to get better transfection efficiency. Actually, viruses have functions to fuse and destabilize the endosomal or lysosomal membrane in their envelopes. For example, adenoviruses have capsid proteins that change their conformation under acidic conditions, such as in the late endosomes, to cause an interaction between the protein and vesicle membrane, disrupting the endosomes. These results suggest that endosomal disruption improves the expression efficiency. Wagner et al. showed that peptide sequences derived from the influenza hemagglutinin HA-2 bound to the transferrin-polylysine molecular conjugate markedly increased the level of transgene expression in cells (Wagner et al. 1992). There have been many attempts to construct synthetic peptides that imitate the endosomolytic functions of viral proteins.

Recently, polyplexes assembled from poly(aspartamide) derivatives bearing 1,2-diaminoethane side chains, [PAsp(DET)], were reported to display amplified *in vitro* and *in vivo* transfection activity (Miyata et al. 2008). PAsp(DET) revealed minimal membrane destabilization at physiological pH, yet there was a significant increase in membrane destabilization at acidic pH, mimicking the late endosomal compartment (pH approximately 5), suggesting the potential for successful transfection.

Nuclear Transport

The final goal of gene delivery is to deliver exogenous DNA to the nucleus, where it can proceed to transcription and translation to get functional peptides or proteins. The molecules are transported between the cytoplasm and the nucleus through the nuclear pore complex (NPC), which is a large protein complex of about 125 MDa composed of 50–100 different proteins. To freely diffuse through the NPC channel, a molecule must be less than 40 kDa, and molecules that are larger than 40 kDa need an active process in order to pass through. Nuclear localization signals (NLS), oligopeptides mainly composed of cationic residues, are used to enhance nuclear trafficking through the NPC. Several viral proteins are known to provide translocation activity of the viral genome to the nucleus.

A great deal of effort has been made to enhance transfection efficiencies with non-viral vectors by conjugating NLS peptides to DNA. Plasmid DNA coupled to the NLS derived from simian virus 40 (SV-40) large T antigen effectively targeted zebrafish embryo nuclei.

THERAPEUTIC GENE

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 in order to maintain cellular function, or to deliver proteins that induce proliferation or apoptosis of the target cells. In cardiovascular medicine, gene therapy has been applied to treat coronary artery disease, peripheral artery disease, restenosis after vascular interventions and graft failure, hyperlipidemia, thrombosis, and pulmonary arterial hypertension (PAH). Table 2 lists therapeutic targets and genes used for gene therapy.

Table 2. Therapeutic target diseases and genes used for gene therapy in cardiovascular disease.

Therapeutic target disease	Treatment genes
Therapeutic angiogenesis	VEGF-A, -B, -V, -D, -E, FGF-1, -2, -4, -5, angiopoetin-1, HGF, MCP-1, PDGF, eNOS, iNOS, adrenomedullin
Restenosis, 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 oligonucleotide ribozimes blocking 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 synthase, VEGF-A, adrenomedullin
Vasospasm after SAH	Endothelial NOS, ECSOD, CuZnSOD, Antisense preproendothelin-1, Prepro-CGRP

VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; eNOS, endothelial nitric oxide synthase; CGRP, calcitonin gene-related peptide.

Coronary Artery Disease and Peripheral Artery Disease

Atherosclerosis is the most prevalent process to affect adult coronary and peripheral arteries. Atherosclerotic lesions reduce the lumen of the arteries, leading to a reduction in the arterial blood supply to the myocardium or skeletal muscle. Stimulation of collateral vessel formation by the use of gene therapy will help to increase perfusion of the ischemic tissues. Gene-encoding growth factors, such as vascular endothelial growth factor, fibroblast growth factor, and hepatocyte growth factor, have been successfully tested in animal models and clinical trials for therapeutic angiogenesis.

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 in the prognosis of peripheral and coronary artery disease. Smooth muscle cell proliferation, remodeling, matrix deposition, thrombosis, and platelet and leukocyte adhesion may play roles in the development of arterial restenosis in these settings. To decrease vascular cell proliferation, various gene therapy strategies have been employed. Anti-proliferative strategies designed for the treatment of experimental cardiovascular disease can be grouped into two main categories: (1) antisense approaches, ribozymes, transcription-factor decoys, and siRNA strategies to inactivate positive cell-cycle regulators; and (2) over-expression of negative regulators of cell growth.

Pulmonary Arterial Hypertension

PAH is characterized by a progressive increase in pulmonary vascular resistance, leading to right heart failure and death. Recent advances in therapeutic approaches to PAH show promising targeting pathways believed to play critical pathogenic or pathophysiologic roles. Despite these findings, PAH remains a challenging condition. The average survival from the time of diagnosis is 2.8 y (Nagaya 2004). To reduce pulmonary vascular resistance, the transfer of genes encoding endothelial nitric oxide synthase, calcitonin gene-related peptide, prostacyclin synthase, and adrenomedullin (AM) has been shown to be effective in animal models (Nagaya et al. 2000; Harada-Shiba et al. 2009).

SELECTION OF ROUTE DEPENDING ON THE TARGET TISSUE

Intravenous Administration

The administration of a DNA complex has been considered a promising way to deliver genes to organs, and many attempts have been reported. However, it is not an easy way to deliver genes successfully because there are many barriers for the gene to go through. For example, the intravenous administration of positively charged polyplex gives aggregation with negatively charged proteins abundantly reserved in the blood. Endothelial barriers are another hurdle on the way to the target. Many attempts have been made to deliver specific organs via specific receptors, as mentioned in the subsections on receptor-mediated gene delivery in the section on development of gene carriers.

Intratracheal Administration

Intratracheal administration can avoid the propensity of nuclease to degrade in the blood compartment. The lung has an enormous surface area that can serve as a therapeutic bioreactor for the delivery of therapeutic genes. Pulmonary administration is a promising therapeutic route, especially in the clinic, by virtue of its high patient compliance with the use of an inhaler or nebulizer.

Special features of pulmonary gene delivery via airways are that the lung has characteristics that critically influence transfection efficiency, such as the presence of surfactant, alveolar macrophages, and mucociliary clearance mechanisms. In the early 1990s, lipoplex was used by aerosol delivery or intratracheal instillation. However, cationic lipids were shown to have decreased transfection efficiency due to interaction with lung surfactants compared to a cationic polymer like PEI. To overcome the surfactant barriers, cationic emulsion was used and showed much higher transfection activity than lipoplexes such as Lipofectin, Lipofectamine, and DMRIE/c. However, even for cationic emulsion, the gene's expression efficiency was limited. Polyplexes and polyplex nanomicelles made from cationic polymer or block copolymer were reported to show higher transfection efficiency than cationic lipoplexes for pulmonary gene delivery via airways.

VECTORS USED IN GENE DELIVERY

Polymer

Synthetic polymer vectors possess cationic charges and form a PIC with negatively charged DNA based on electrostatic interactions. The transfection efficacy depends on their chemical structure, charge density, and molecular weight. The list of polymer vectors is shown in Table 3.

Polyethyleneimine (PEI)

PEI, a polycation with high ionic charge density, has been used as a gene delivery material. PEI binds to DNA by an electrostatic interaction to form small complexes that are internalized into cells and can localize to the nucleus. PEI's high transfection efficiency can be caused by its large buffering capacity, which may change endosomal osmolarity. Endosomal release may enhance gene delivery to the nucleus (Yamagata et al. 2007). Intravenous delivery of PEI is not suitable for targeting of the lung. PEI

Table 3. List of polymer vectors.

Polymers	Modified polymers
Block copolymer	PEG-poly(aspartate) (PEG-PAsp) PEG-poly(glutamate) (PEG-PGlu) PEG-poly(lysine) (PEG-PLys) PEG-chitosan PEG-b-P[Asp(DET)]
PLGA poly(lactic-coglycolic acid)	Chitosan-modified PLGA PEG-PLGA
Chitosan	Glycol chitosan PEI-graft-chitosan chitosan lactate
PEI poly(ethyleneimine)	Branched PEI Galactose-PEG-PEI Glucosylated PEI Cell-penetrating peptides-PEG-PEI
Poly-L-lysine	
Dendrimer	G2/G3 polyamidoamine (PAMAM) G9 PAMAM

or modified PEI has been shown to be one of the most effective agents for constructing gene delivery systems available today, with high levels of pulmonary gene transfer via the airways (Densmore 2006).

Chitosan

Chitosan is produced by the deacetylation of chitin, which is the structural component in the exoskeleton of crabs and shrimp. Chitosan is also a promising biopolymer as a carrier material because of its biocompatibility, low toxicity, and low degradation activity. This product is made at relatively low cost because natural sources of it are abundant. Though complexes of chitosan-DNA are stable against DNase, the transfection efficiency of chitosan-DNA is generally lower than those of other non-viral vectors. The degree of deacetylation, molecular weight, and N/P ratio (the ratio of the number of nitrogen molecules in chitosan against that of phosphate in a gene) are effective factors for controlling the gene delivery efficiency.

Dendrimers

Dendrimers are typically symmetric around the core and are composed of hyperbranched amino-acid units. Dendrimers are classified by generation, which refers to the number of repeated branching cycles that are performed during their synthesis. In general, dendrimers form a neutral complex with plasmid DNA or siRNA; they have properties by which free DNA is released from the complex more easily than from other polymers, thus providing high transfection efficiency.

Liposome

Cationic liposomes condense DNA to form particles (100 to 200 nm), called lipoplexes, based on electrostatic interactions and protect the DNA from degradation. In most cases, after lipoplexes are intravenously injected, the highest levels of gene expression are obtained in the lung because the lung capillaries are the first traps. Cationic liposomes have been clinically evaluated and are popular carriers for gene delivery to the lungs (Desmore 2006).

Liposomes conjugated with cell-penetrating peptides are used for intracellular delivery to the lung because they enhance the liposome uptake by cells. Pulmonary gene delivery via inhalation has an advantage in that the lung has critical features influencing transfection efficiency, such as the presence of alveolar surfactant, macrophage, and mucociliary clearance mechanisms. Lipoplexes were used with aerosol delivery or intratracheal instillation in the early 1990s. However, in gene delivery, cationic lipids were shown to have reduced transfection efficiency due to interaction with lung surfactants compared with a cationic polymer like PEI (Wiseman et al. 2003; Bragonzi et al. 1999).

Polyplex Nanomicelles

To overcome the drawbacks of positively charged DNA complexes that bind to proteins and erythrocytes and form aggregates that are cleared by the reticulo-endothelial system, we have developed a PIC with a cationic block copolymer possessing a hydrophilic segment, PEG (Fig. 1). The complexes of DNA and block copolymers form self-assembling particles with core-shell structures called PIC nanomicelles. PIC nanomicelles are water-soluble and nuclease-resistant nanoparticles with small zeta potential (Katayose et al. 1998). These nanomicelles show high colloidal stability under physiological conditions and substantial transfection activity against various cell types even after pre-incubation with serum proteins. Nanomicelles remained intact for 3 h after intravenous administration, suggesting high stability in circulating blood (Harada-Shiba et al. 2002).

High transfection efficiency and low cytotoxicity with the use of PIC nanomicelles formed by PEG-block-poly(aspartamide) copolymers carrying the N-(2-aminoethyl)-2-aminoethyl group in the side chain (PEG-P[Asp(DET)]) were reported (Kanayama et al. 2006). These successful *in vivo* gene therapies have been explained by the specific structure of the side chain of P[Asp(DET)], in which the 1,2-ethanediamine moiety of N-(2-aminoethyl)-2-aminoethyl group exhibits distinct two-step protonation behavior, suggesting the potential proton sponge capacity of Asp(DET) units for efficient endosomal escape.

IN VIVO GENE DELIVERY BY INTRATRACHEAL ADMINISTRATION USING POLYPLEX NANOMICELLES FOR TREATMENT OF ANIMAL MODELS OF PAH

The large number of human diseases presenting poor prognoses and limited efficacy under current therapeutic regimens necessitates the development of alternative approaches. PAH is such a disease that lacks a highly efficacious therapeutic regimen. PAH patients are currently treated with a variety of drugs, including prostacyclin, prostacyclin analogs, calcium channel blockers, nitric oxide (NO) inhalation, angiotensin-converting enzyme inhibitors, endothelin receptor antagonists, and phosphodiesterase 5 inhibitors; in severe cases, lung transplantation and subsequent immunosuppression are necessary. Viral or viral-related vectors have been used for the delivery of therapeutic genes, and these gene carriers have the potential for immunogenicity and inflammatory response. In diseases where a single dose can cure or provide palliative care, viral vectors may be suitable. However, PAH therapy requires repeated administration for efficacy, hence the utility of viral or viral-based gene therapy is contraindicated.

P[Asp(DET)], a poly(aspartamide) derivative bearing an N-(2-aminoethyl)aminoethyl group as the side chain, showed improved transfection efficiency and biocompatibility compared to linear poly(ethyleneimine) (LPEI) (Kanayama et al. 2006). PEG-b-P[Asp(DET)] was applied to the *in vivo* delivery of therapeutic plasmids for a murine, skull bone defect model and a rabbit carotid artery with neointima model; its successful therapeutic efficacy in these mammalian studies provided the impetus for expanded application to gene therapy for intractable diseases (Itaka et al. 2007; Akagi et al. 2007).

We applied the gene delivery system to introduce genes into mice and found a 100-fold increase in transgene expression by the intratracheal administration of PEG-b-P[Asp(DET)] compared with LPEI (Fig. 4) (Harada-Shiba et al. 2009). The high levels of gene expression persisted for 14 d. PEG-*b*-P[Asp(DET)] polyplex nanomicelles loaded with pDNA bearing the yellow fluorescence protein (YFP) gene (N/P = 80) or LPEI/pYFP polyplexes (N/P = 6) were sprayed intratracheally into mice. The results showed that significantly higher fluorescence intensity was clearly seen in the lungs treated with the PEG-*b*-P[Asp(DET)] polyplex nanomicelle than in the LPEI polyplex controls. The lungs administered with LPEI/pLuc showed moderate infiltration of neutrophils at the terminal bronchiole and alveoli. However, in the lungs administered with PEG-*b*-P[Asp(DET)]/pLuc, neutrophilic infiltration was scattered and either minimal or absent, suggesting increased biocompatibility with the

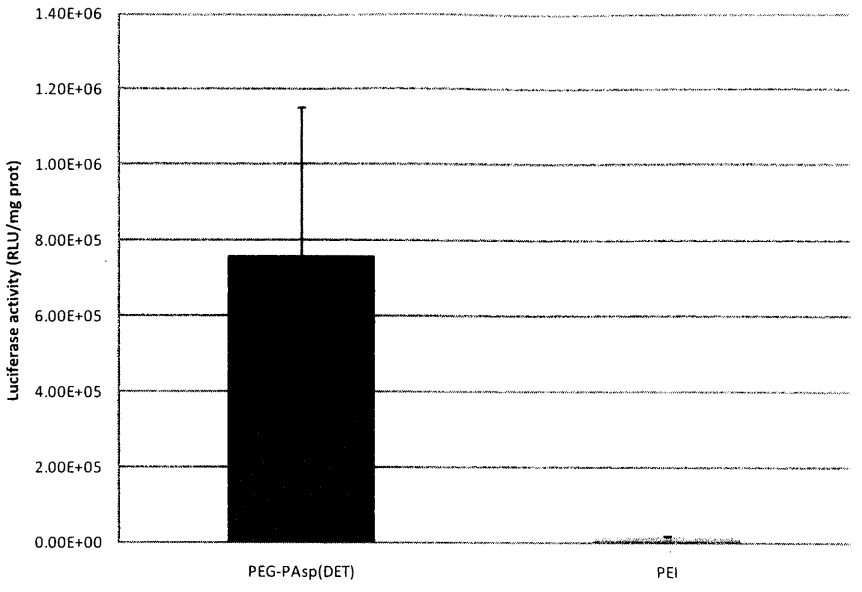

Fig. 4. Luciferase gene expression by intratracheal administration of LPEI polyplex (N/P = 6) or PEG-*b*-P[Asp(DET)] polyplex nanomicelle (N/P = 80). Samples of the polyplex and the polyplex nanomicelle were prepared before the administration and left for 1 d. The mice (five per group) were anesthetized and the polyplex or the polyplex nanomicelle was administered intratracheally. At 24 h post-administration, the lung tissues were harvested, homogenized, and measured for luciferase activity (mean + SEM, N = 5). With permission from Harada-Shiba et al. (2009).

PEG-*b*-P[Asp(DET)] polyplex micelle. Pro-inflammatory cytokine mRNA levels did not increase for intratracheally administered naked pLuc in saline or for the PEG-*b*-P[Asp(DET)] polyplex micelles. However, LPEI/pLuc polyplexes revealed a two-fold increase in tumor necrosis factor α, IL-6, 1L-10, and Cox-2 compared to the control, suggesting that polyplex nanomicelles have favorable properties for *in vivo* use.

Then, a PEG-*b*-P[Asp(DET)] polyplex nanomicelle system was tested to introduce the AM gene via intratracheal administration in PAH model rats. After 4 wk of monocrotaline injection, right ventricular pressure was increased to twice the normal value. Notably, right ventricular pressure was decreased significantly by an intratracheal spray of the PEG-*b*-P[Asp(DET)] polyplex nanomicelle loaded with the expression vector of AM (N/P = 40) (Fig. 5). On the other hand, right ventricular pressure did not change significantly after administration of naked plasmid encoding the AM gene in saline or the LPEI polyplex loaded with the AM gene, or with the administration of the polyplex nanomicelle loaded with the luciferase gene. The lungs transfected with the polyplex nanomicelle

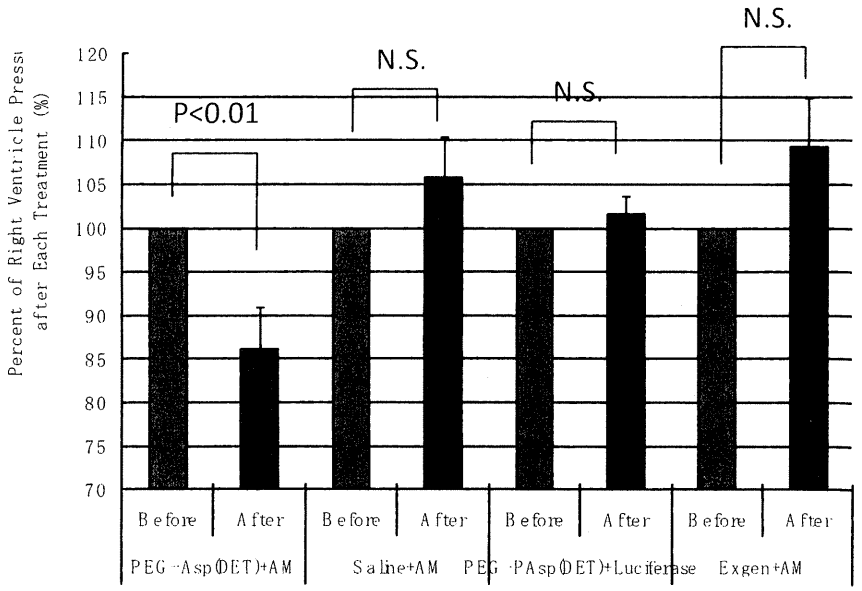

Fig. 5. Effect of gene transfer on right ventricle pressure in the PAH rat model. The PEG-*b*-P[Asp(DET)] polyplex nanomicelle loaded with AM expression vector; AM expression vector in saline; PEG-*b*-P[Asp(DET)] polyplex micelle loaded with luciferase gene; or the LPEI polyplex loaded with AM expression vector was sprayed intratracheally. Three days later, a hemodynamic study was performed again to measure the right ventricle pressure, with the results indicated as "After". With permission from Harada-Shiba et al. (2009).

loaded with the AM expression vector had high levels of AM mRNA. Alternatively, the levels were much lower in the lungs transfected with the LPEI polyplex loaded with the AM expression vector. These results suggest that successful delivery of the therapeutic gene was performed by intratracheal administration using PEG-*b*-P[Asp(DET)] polyplex nanomicelle.

APPLICATIONS TO OTHER AREAS OF HEALTH AND DISEASE

Intratracheal administration of therapeutic genes using PEG-*b*-P[Asp(DET)] polyplex nanomicelle can be applied to lung diseases such as cystic fibrosis, alpha 1-antitrypsin deficiency, asthma, or lung cancer. It can also be applied to other diseases for the lungs to use as a reservoir organ.

Summary Points

- To successfully deliver genes to target tissue, three primary factors must be addressed: the development of an appropriate gene carrier, the selection of a therapeutic gene, and the selection of an administration route.
- For successful gene delivery to target cells, carriers should provide properties that can break through four major barriers: (1) a system that can protect plasmids from the environment during the route to target cells, (2) a system that can be incorporated into the target cells, (3) a system that can enable endosomal escape, and (4) a system that can be transported to the nucleus.
- In cardiovascular medicine, gene therapy has been applied to treat coronary artery disease, peripheral artery disease, restenosis after vascular interventions and graft failure, hyperlipidemia, thrombosis, and pulmonary arterial hypertension.
- Development of non-viral vectors such as polymer, block copolymer, and liposome have been developed and used for the treatment of animal models as well as patients.
- A therapeutic gene was successfully delivered by intratracheal administration using a PEG-*b*-P[Asp(DET)] polyplex nanomicelle, which is water soluble and nano-sized, has low zeta potentials, and can act as a proton sponge for efficient endososomal escape. We succeeded in the treatment of PAH animal models by delivering the AM gene using the PEG-*b*-P[Asp(DET)] polyplex nanomicelle without adverse effect.

Key Facts

- Plasmid DNA needs to be compacted and condensed in order to remain intact during the route to the target cells.
- Cationic block copolymer, consisting of hydrophilic PEG and cationic polymer, produces DNA condensate through the formation of polyion complex (PIC) micelles.
- Gene delivery can be targeted by using receptor-mediated delivery.
- The endosomal escape step is recognized as the biggest barrier to successful gene delivery and expression.
- Nuclear localization signals, oligopeptides mainly composed of cationic residues, are used to enhance nuclear trafficking.

- In cardiovascular medicine, gene therapy has been applied to treat coronary artery disease, peripheral artery disease, restenosis after vascular interventions and graft failure, hyperlipidemia, thrombosis, and pulmonary arterial hypertension.
- Pulmonary administration of a gene is a promising therapeutic route of administration, especially in the clinic, because patients show high degrees of compliance with the use of inhalers or nebulizers.
- The complexes of DNA and block copolymer form self-assembling particles with core-shell structures, PIC nanomicelles.
- PIC nanomicelles have high transfection efficiency and low toxicity.
- Successful delivery of the adrenomedullin gene was performed by intratracheal administration using PEG-*b*-P[Asp(DET)] polyplex nanomicelle, ameliorating pulmonary arterial hypertension in rat models.

Definitions

AM: The most potent peptide that relaxes smooth muscle cells.

Endosomal escape: A property that non-viral vectors should have to get better expression of genes, which are the mechanisms that are not degraded in the endosome.

Lipoplex: A complex consisting of a liposome and a gene.

Nuclear pore complex: A complex of proteins on the surface of the nucleus that plays a role in the gate of the nucleus.

Polyplex nanomicelle: A complex consisting of a block copolymer and a gene.

Polyplex: A complex consisting of a polymer vector and a gene.

Transfection: Introduction of the genes to the cells.

Abbreviations

AM	:	adrenomedullin
DDS	:	drug delivery systems
IL	:	interleukin
LDL	:	low-density lipoprotein
LPEI	:	linear polyethyleneimine
N/P ratios	:	nitrogen/phosphate ratios
NLS	:	nuclear localization signal
NO	:	nitric oxide
NPC	:	nuclear pore complex
PAH	:	pulmonary arterial hypertension
PEG	:	polyethylene glycol

PEG-*b*-P[Asp(DET)]	:	PEG*b*-poly{*N*-[*N*-(2-aminoethyl)-2-aminoethyl]aspartamide}
PEI	:	polyethyleneimine
PIC	:	polyion complex
SV-40	:	simian virus-40
YFP	:	yellow fluorescence protein

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23

Inhibition of Tumor Growth and Metastasis by a Combination of Anti-VEGF-C and Enhanced IL-12 Therapy in an Immunocompetent Mouse Mammary Cancer Model

Masa-Aki Shibata[1], Junji Morimoto[2], Eiko Shibata[3], Mariko Harada-Shiba[3] and Shigekazu Fujioka[1]

[1]*Laboratory of Anatomy and Histopathology, Faculty of Health Science, Osaka Health Science University, Temma, Osaka*

[2]*Laboratory Animal Center, Osaka Medical College, Osaka*

[3]*Department of Molecular Innovation in Lipidology, National Cerebral & Cardiovascular Center Research Institute, Osaka*

Japan

1. Introduction

Breast cancer represents a major health problem in women, with more than 1,000,000 new cases and 370,000 deaths yearly worldwide [1]. Perhaps more worrisome is an apparently increasing incidence of breast cancer among younger women under 40 years of age recently reported in many countries worldwide [2-4]. The lethality of breast cancer is largely due to metastasis, preferentially to the lymph nodes, lungs and bones [5]; in order to delay the progression of breast cancer and prolong patient life, more effective chemopreventive and antimetastatic treatments and less toxic chemotherapeutic agents are desperately required.

Vascular endothelial growth factor-C (VEGF-C) is expressed in a variety of malignant tumors including mammary cancer [6] and over-expression of VEGF-C has been reported to be associated with lymph node metastasis and poor prognosis in breast cancer patients [7,8]. A number of animal studies using cell lines [9-11] and transgenic mice[12] have been conducted in an attempt to demonstrate that VEGF-C over-expression is able to promote cancer metastasis. Using a 'RNA interference' approach with an immunocompetent mouse mammary cancer model, we previously demonstrated that inhibition of VEGF-C or VEGF-A by gene silencing using vectors expressing short interfering RNA (siRNA) leads to suppression of lymphatic and/or hematogenous metastasis [13].

The cytokine interleukin-12 (IL-12), a heterodimer composed of p35 and p40 subunits, is produced primarily by dendritic cells, macrophages/monocytes, and neutrophils and functions in enhancing the activity of cytotoxic T lymphocytes and NK cells. Both subunits are necessary to exert biological activity [14]. IL-12 plays an important role in the induction of a cell-mediated immune response [15]. This cytokine is also involved in the differentiation of native T cells to the Th1 subset, and induces production of interferon-γ (IFNγ) in both T and NK cells. In addition, IL-12 has been shown to exert a potent anti-neoplastic effect in a