preferentially expressed in tumor endothelium *in vivo* and that its expression is regulated by tumor-derived factors. Now, it is highlighted that endocan is a marker of EC activation during growth of the new vessels required for tumor progression [46].

4.2.3. Decorin. Decorin, a member of the small leucine-rich repeat proteoglycan (SLRP) family, is expressed by sprouting ECs during inflammation-induced angiogenesis *in vivo* and by human ECs cocultured with fibroblasts in a collagen lattice. Activation with IL-10 or IL-6 treatment induces decorin mRNA in human ECs [47]. As function of decorin in human ECs, it has been reported that decorin core protein can bind to and activate insulin-like growth factor-I receptor (IGF-IR) [48] and that decorin promotes $\alpha 2\beta 1$ integrin-dependent EC adhesion and migration on fibrillar collagen type I [49]. It is now understood that modulation of cell-matrix interactions by decorin plays a key role during angiogenesis [50].

4.2.4. Versican. Primary human ECs, if stimulated with TNF-α or VEGF, alter their expression of versican (a large aggregating CSPG) by *de novo* transcription of the V3 isoform and by exhibiting a moderate V1/V2 production. Induced versican synthesis and *de novo* V3 expression were also observed in ECs induced to migrate in a wound-healing model *in vitro* and in angiogenic ECs forming tubule-like structures in Matrigel or fibrin clots. Thus, in activated conditions, versican expression in human ECs is altered [51]. This study indicates that versican produced from ECs plays a key role in the pathological conditions such as inflammation, angiogenesis, and wound healing.

4.2.5. Biglycan and PG-100. When EA.hy 926 cells, one of human ECs, form monolayer cultures typical of macrovascular ECs, they express and synthesize detectable amounts of biglycan and PG-100 (members of the small leucine-rich repeat proteoglycan family). TNF-α, responsible for changing the morphology of the cells from a polygonal to a spindle shape and for stimulating the detachment of the cells from the culture dish, markedly decreased the synthesis of biglycan. By contrast, PG-100 expression was increased in response to FGF-2, FGF-7, TNF-α, and TGF-β [52]. Although the functional roles of biglycan and PG-100 are not yet clearly understood, their different responses to the stimuli may be critical for the progression of vascular diseases. Another study has shown that antiangiogenic antithrombin treatment significantly decreases biglycan in HUVECs [42], suggesting that a mechanism of antiangiogenic antithrombins is through the downregulation of proangiogenic biglycan.

4.3. Cell Surface PGs

4.3.1. Glypicans. Glypican-1 is the only glypican expressed in the vascular system. VEGF165 interacts with EC-derived glypican-1 dependent on HS [53]. Recently, the contribution of glypican-1 to the cell cycle and proliferation has been demonstrated in ECs [54].

4.3.2. Syndecans. Proteoglycans (PGs) are important constituents of the plasma membrane and of the basement membrane supporting the EC layer. Changes in the amounts or the structures of PGs in the endothelium may affect important functions, such as turnover of lipoproteins, filtration properties, and regulation of chemokines during inflammation, which are all relevant to diabetes. In HUVECs, exposure to high glucose (hyperglycemic condition) leads to decreased secretion of syndecan-1 [55]. Thrombospondin-1 (TSP-1), an extracellular matrix protein, modulates focal adhesion in mammalian cells and exhibits dual roles in angiogenesis. There are indications that binding of TSP-1 to syndecan-4 proteoglycan mediates tubulogenesis and their protection from apoptosis [56]. Syndecan-2, the major syndecan expressed by human microvascular ECs (HMECs), is regulated by growth factors and extracellular matrix proteins, in both bidimensional and tridimensional culture conditions [57]. Downregulation of syndecan-2 reduced the spreading and adhesion of HMECs, and it not only enhanced their migration but also impaired the formation of capillary-like structures. Therefore, syndecan-2 has an important function in some of the necessary steps in the angiogenic process. Syndecan-1 is a critical regulator of $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins during angiogenesis and tumorigenesis, and it is inhibited by the novel peptide called synstatin [58].

5. Glycoconjugate-Related Molecules: Galectins

Galectins are a family of lectins that bind to β-galactosides via a carbohydrate recognition domain containing many conserved sequence elements [59]. There are currently 15 known mammalian galectins [60], which are involved in a variety of biological processes [61]. Expression of galectin-1 in cultured human ECs was first shown in 1995 by Baum et al. [62]. They also showed that activation of cultured ECs by minimally oxidized low-density lipoprotein (MM-LDL) or cytokines and LPS increases galectin-1 expression, as determined by ELISA, northern blot analysis, and high-throughput cDNA sequencing. Another study found that poly IC, a synthetic analog of double-stranded RNA (dsRNA), enhances the expression of galectin-9 mRNA and protein in ECs in concentration- and time-dependent manners [63]. Treatment of cells with dsRNA *in vitro* mimics viral infection and regulates the expression of various genes. Thus, it has been proposed that upregulation of galectin-9 expression by poly IC in the vascular endothelium may be part of the mechanism for leukocyte trafficking through the vascular wall after viral infection. Subsequently, galectin-1, -3, -8, and -9 expression levels in quiescent ECs were measured, and the expression and distribution of these galectins changed after activation with tumor-derived culture medium [64]. Recently, it was reported that galectin-9 protein expression is positively regulated by histone deacetylase 3 in ECs [65]. This study provides new evidence that HDAC3 regulates galectin-9 expression in ECs via interaction with the PI3K-IRF3 signaling pathway.

Some functional analyses of galectins in human ECs have been reported. Cancer-associated carbohydrate T antigen

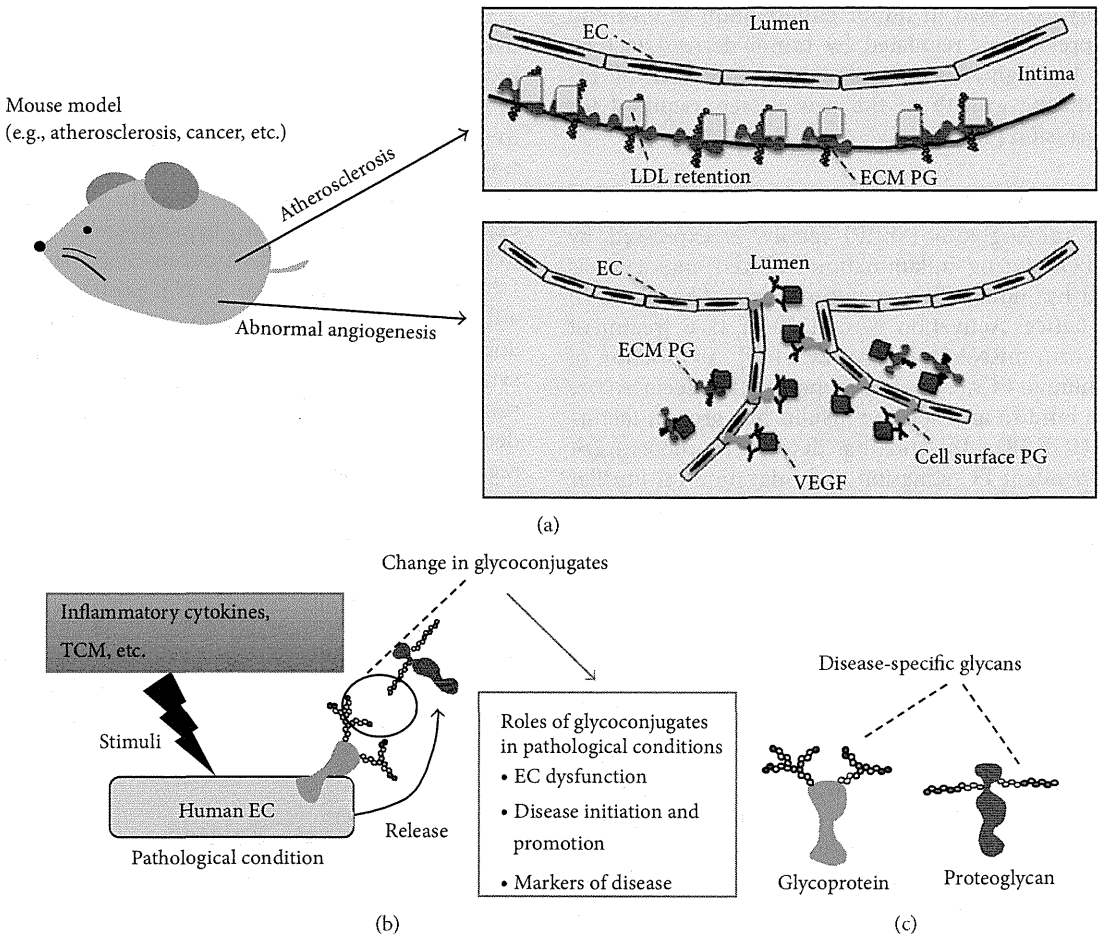

FIGURE 2: Glycoconjugates and peripheral artery disease (PAD). (a) The contribution of glycoconjugates to vascular-related diseases has been examined in mouse models. Initiation and promotion of atherosclerosis via interaction between LDL and proteoglycans (PGs) have been indicated. Additionally, the importance of the interaction between growth factors (e.g., VEGF165) and heparan sulfate PG in ECs and extracellular matrix (ECM) during abnormal angiogenesis, such as in tumorigenesis, has been shown. (b) In human ECs, pathological stimulation, such as inflammatory cytokines and tumor-cell-derived medium (TCM), induces changes in glycoconjugates (e.g., expression levels, glycan structures, etc.). These changes may lead to EC dysfunction and disease initiation and promotion. Furthermore, glycoconjugates specifically modified under pathological conditions may be candidates for markers of PAD. (c) Study of human ECs to identify specific glycoconjugates related to PAD may be a good strategy for the prevention and treatment of PAD.

plays a leading role in docking breast and prostate cancer cells onto endothelium by specifically interacting with EC-expressed galectin-3 [66]. One of the low-molecular-weight synthetic lactulose amines (SLAs), *N*, *N'*-dilactulose-octamethylenediamine (D-LDO), severely impaired tube formation of ECs. This inhibitory effect is thought to occur by inhibition of galectin-1- and/or galectin-3-mediated functions. D-LDO inhibited the binding of galectin-1 and/or galectin-3 to the highly glycosylated protein 90 K. Thus, D-LDO may be a new galectin inhibitor for blocking angiogenesis [67]. In HUVECs, knockdown of galectin-3 and Mgat5, an enzyme that synthesizes high-affinity glycan ligands of galectin-3, reduced VEGF-A-mediated angiogenesis *in vitro*. A direct interaction was detected on the plasma membrane between galectin-3 and VEGF-R2, and this interaction was dependent on the expression of Mgat5. Using immunofluorescence and cell surface labeling, an increase in the level of internalized VEGF-R2 was observed in both Mgat5 and galectin-3 knockdown cells, suggesting that galectin-3 retains the receptor on the plasma membrane via lattice formation. Thus, galectin-3 contributes to the plasma membrane retention and proangiogenic functions of VEGF-R2 in ECs [68].

6. Glycan-Degrading Enzymes

6.1. Heparanase. Heparanase is an endo-β-D-glucuronidase responsible for heparan sulfate (HS) degradation at a limited number of sites, yielding HS fragments of an appreciable size (5–7 kDa) and with biological potency [69, 70]. In 1991, heparanase activity was observed in human ECs [71]. Few reports of functional analyses of endogenous heparanase have since been published. The HS content of the endothelium is reduced under hyperglycemic conditions, and it may contribute to the pathogenesis of atherosclerosis. This suggests that HS reduction in ECs is due to increased heparanase production under hyperglycemic conditions.

Recently, high-glucose-induced heparanase production and HS degradation were detected in human several types of ECs [72, 73]. In fact, functional analyses revealed that the expression of heparanase contributed to EC migration and proliferation. Furthermore, it has been shown that EC proliferation and migration correlate with Akt and ERK phosphorylation levels [72].

6.2. Sialidase. Sialidases are glycosidases that catalyze the removal of α-glycosidically linked sialic acid residues from the carbohydrate groups of glycoproteins and glycolipids. To date, at least 4 mammalian sialidases, NEU1–4, have been identified [74, 75]. Whether the EC surface expresses sialidase activity was not known until recently. In human lung microvascular ECs (HMVEC-Ls) and human pulmonary artery ECs (HPAECs), NEU1–4 expression was examined, and functional analysis by knockdown and overexpression was performed. These ECs express catalytically active NEU1 and NEU3 sialidases, and NEU1 restrains the endothelial migratory response to wounding. It was concluded that NEU1 regulates endothelial remodeling in response to injury [76].

7. Closing Remark

In this paper, we described previous research about glycoconjugates in human ECs. To date, the functional roles of glycoconjugates *in vivo* have been investigated in animal disease models (e.g., atherosclerosis) (Figure 2(a)). In apoE-null mice, HS of perlecan contributes to the promotion of atherosclerosis [77]. This contribution may involve increased LDL retention, altered vascular permeability, or other mechanisms. Additionally, a proatherosclerosis function of biglycan has been suggested by the retention of LDL [78]. Abnormal angiogenesis plays a critical role in the pathogenesis of many diseases such as cancer, ischemic vascular disorders, and diabetic retinopathy [79]. In particular, the importance of HS in tumor angiogenesis has been demonstrated by tumor-based studies in mice with endothelial-targeted genetic alterations in HS biosynthesis (Figure 2(a)) [80]. Thus, the functional importance of glycoconjugates in vascular-related diseases has been demonstrated.

Glycoconjugates are markers for cell context, and they play biological roles. Increasing evidence from examination of human ECs indicates that glycoconjugates also serve as biomarkers and functional players in the human endothelium under pathological conditions as described here (Figure 2(b)). Therefore, although many issues await clarification, glycoconjugates are attractive potential targets for the prevention and treatment of vascular-related diseases. In recent years, the incidence of vascular-related diseases, such as cancer, cardiac disease, and cerebrovascular disease, has increased with an aging population, and prompt countermeasures are sought. Hence, further study of the functional roles of glycoconjugates in human ECs and identification of specific glycoconjugates related to disease are required in order to develop therapeutic strategies for vascular-related diseases, such as peripheral artery disease (PAD) (Figure 2(c)).

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再生医療に活かせるstem cell（SC）研究の進歩*

豊田雅士**　梅澤明弘***

はじめに

ヒトの体（個体）は器官の集合体であり，臓器，さらには細胞から構成されている。そして再生医療とはもともと事故や病気によって失われた体の細胞，組織，器官の再生や機能の回復を目的とした医療である。その意味から捉えると，運動学などを活かしたリハビリテーション，義肢や人工関節，人工血管といった人工材料を用いた工学的アプローチによる治療，皮膚移植や骨髄移植，また臓器移植といった生きた細胞や臓器を使った移植医療なども再生医療といえる。しかし今では，本人もしくは他人の幹細胞を培養などで加工し，失われた組織や臓器を修復・再生させる医療，すなわち幹細胞移植医療が「再生医療」であるとの認識が強くなっている。これは，「多能性幹細胞は体を構成するあらゆる細胞・組織に分化する能力」があり，また「組織幹細胞は体のあらゆるところに存在する」ことがわかりつつあり，これまで治療が難しいとされたさまざまな疾患に対する新たな医療を提供することが可能になると期待されているからに違いない。では再生医療のソースとしての幹細胞はいったいどれくらいの種類があるのであろうか（図1）。本稿では，再生医療に活かせる幹細胞（図2）とはどのようなものがあり，それがどんな特性をもち，実際に臨床で使えるようになるかを考えてみたい。

I 再生医学と幹細胞

プラナリアという小さな生き物は，体のあらゆるところを切断しても1個体として再生し，またザリガニやイモリなどの生物種では肢や心臓，眼など一部の組織において再生能力をもっている。一方ヒトは，皮膚や血液などは日々の新陳代謝のなかで再生しているものの，手足や臓器の丸ごとの再生はできない。進化の過程でなぜヒトが再生能力を失ったかの謎はいまだ解けていないが，再生研究を医療へ応用し治療に利用していくことを目指す再生医学は著しい進展をみせている。1970年代にヒトの軟骨や皮膚から細胞を培養することに成功し，さらに骨髄中に血液の元となる造血幹細胞が同定されると，それを用いた移植医療が行われ白血病などの治療に効を奏するようになる。これは疾患や事故によって失われたさまざまな生体機能が，幹細胞によって再生・治療できることを示すこととなった。その幹細胞の存在は，ヒトの発生過程における器官形成となる胎児期に限定され，成人にはほとんどないといわれてきたが，多くの幹細胞が存在しヒトの生体内における恒常

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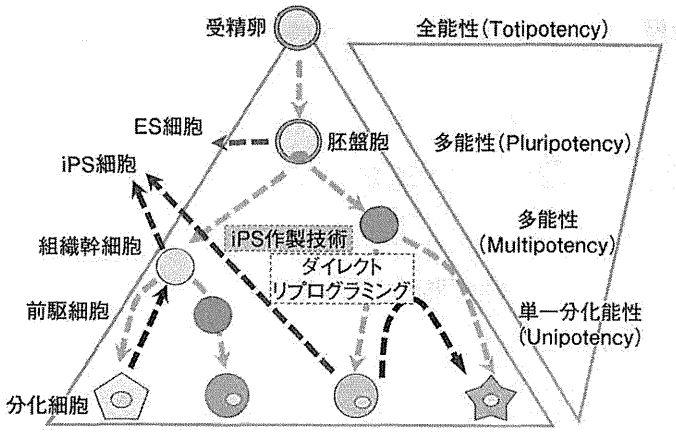

図1　さまざまな幹細胞と分化能

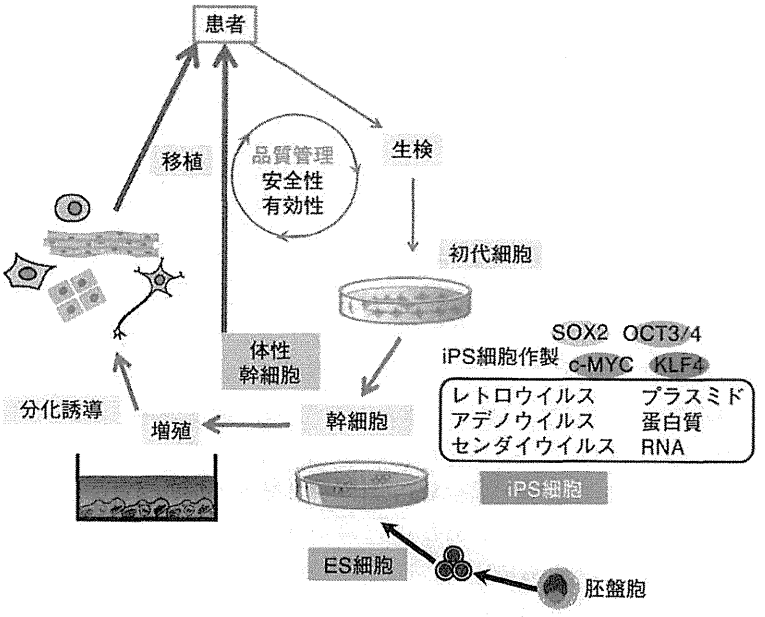

図2　幹細胞による再生医療

性維持に関与していることが明らかになりつつある。

I 胎児期由来の幹細胞

ヒトの胎児期は機能的な器官形成が行われる時期であり，その時期の細胞（幹細胞）・組織を取り出し，さまざまな疾患に対する移植治療に用いることができるのではないかという考えは以前よりあった。特に1980年代後半以降，中絶胎児から得られたドーパミン産生細胞を移植するパーキンソン病に対する治療は盛んに行われ，一部で効果が認められたとする報告もある。しかしこのような細胞移植には，十分な治療効果を得るために多数の中絶胎児を必要とするなど倫理的な問題に対する議論があり，一般的治療となるのは難しいと考えられる。

1998年に受精卵が数十回程度分裂した胚（胚盤

胞）に含まれる将来の胎児を形成していく内部細胞塊から「胚性幹細胞（ES 細胞：embryonic stem cell）」の樹立が報告され，また胎児の始原生殖細胞からは胚性生殖細胞（EG 細胞：embryonic germ cell）の樹立が報告された。このヒト ES 細胞と EG 細胞の樹立は，ヒトを構成するあらゆる組織になる多能性を有する可能性を秘め，医療への応用に大いに注目された。しかしここでも胎児期由来の細胞を使うということに対して倫理的な議論が起こり，それは現在でも続いている。さらに移植医療に使用するという実際面においても，移植すれば他人の細胞を受け入れることで起こる免疫拒絶の問題，目的細胞へ確実に分化誘導する方法が確立されていないため，奇形腫形成能を有する未分化 ES 細胞や目的外に分化した細胞を確実に除去していくことが求められるなど，安全性について解決すべき問題がある。しかしこうした課題を抱えながらも，ES 細胞は遺伝子改変などの人為的操作をすることがなく多能性を維持したままほぼ無限に増やすことができることから，さまざまな疾患に対する治療に対応でき，移植に必要とされる細胞数を安定的に確保することが可能であることは大きな利点である。そのため実際に ES 細胞を用いた臨床研究は，これまで治療が難しいとされている難治性疾患である脊髄損傷や網膜色素変性症に対して欧米で実施されている。このうち網膜色素変性症に対する ES 細胞由来の神経細胞移植においては，症状の改善効果が認められたとの報告[1]があり，脚光を浴びた。日本でも倫理面での議論と並行して ES 細胞を使った難治性疾患への治療が計画されるなど，今後新たな展開へと進んでいくことが期待されている。

III 多様な体性幹細胞

成人期の体内で自己複製し，一定の多分化能を保つ幹細胞を「体性幹細胞（somatic stem cell）」という。生体内で起こるさまざまな状況に応じて起こる組織の再生・修復に体性幹細胞が関与し，いわゆる恒常性維持に重要な役割を担っていると考えられている。しかし体性幹細胞の多くは，組織や臓器のどこに存在するのかが厳密に特定されていない。一般には取り出した組織をバラバラにし，単一細胞ごとに培養する。その後，クローンから得られた細胞に多分化能や自己複製能が確認された場合に，幹細胞として同定される。体性幹細胞はもともとごく微量しか存在しないので，分離して得られるものはさらにわずかな量となる。こうした細胞を培養していくのは意外に難しい。

そうしたなかで幹細胞の存在場所として注目されたのが，骨髄である。骨髄中にはもともと造血幹細胞があることが知られていたが，接着性の間質細胞もある。この骨髄間質はさまざまな細胞より構成され，造血幹細胞の維持，分化をコントロールする働きが知られている。1999 年にヒト骨髄間質にある細胞が骨，軟骨，脂肪に分化する多分化能を有する間葉系幹細胞を同定したという報告がされた。その後，この幹細胞が驚くほどの可塑性を有することがわかり，拒絶などの問題のない自分の細胞で自分の組織再生を行う細胞として注目されるようになった。もともと間葉系細胞は骨，軟骨，脂肪，骨格筋，真皮といった結合組織にある細胞の総称であり，細胞外基質を自分で合成して自分の環境を整える能力も強く実質細胞を支持する役割をもっている。こうした間葉系細胞は発生学的にみれば体全体に広がっており，実際骨髄以外にも臍帯，胎盤，月経血，子宮内膜，真皮，脂肪，歯髄，滑膜などから幹細胞が得られたとの報告がある。それぞれの組織・臓器由来の間葉系幹細胞は形態学的によく似ており，幹細胞マーカーの発現様式もほぼ同じであるが，遺伝子発現レベルや分化能に違いが認められる。例えば月経血由来や胎盤組織の一部から得られた間葉系細胞は，骨格筋や心筋に分化するものの脂肪や骨への分化を示さない。こうした間葉系幹細胞についてはこれまでにさまざまな研究機関から報告があり，実際に臨床で最も使われている。しかし骨髄由来や脂肪由来間葉系幹細胞ですら，その採取法や細胞の規格が統一されていないという現実がある。これは間葉系幹細胞が，ES 細胞とは違いへ

テロな集団であるがゆえであるが，再生医療を促進していくためには，まさに今その基準が問われているところでもある[2]。

Ⅳ 人工多能性幹細胞（iPS 細胞：induced pluripotent stem cell）の登場と新たな医療

2006 年に山中らにより報告された iPS 細胞は，これまでの発生学の概念を大きく変えた。多能性を有する幹細胞が最終分化した細胞から得られたことで，ES 細胞が抱える倫理的課題や移植による免疫拒絶の問題をクリアでき，再生医療への期待が一気に加速することとなった。iPS 細胞は ES 細胞で培った培養技術をそのまま用いることができたことや倫理的ハードルが低かったため，より多くの研究機関で培養されたことで研究のスピードが加速されることになった。これまで治療が難しい疾患に対する治療戦略として位置づけられた再生医療を目的として，ゲノムへの挿入がなく安全性が高い作製技術や高効率で iPS 細胞ができる作製法[3]，対象となる細胞（神経，心臓，肝臓，膵臓など）への効率的かつ簡便な分化誘導法，培地や培養皿を工夫し移植するうえで安全な培養法，未分化細胞を除去するための方法などが次々に開発されてきている。iPS 細胞による再生医療の実現化には，作製時に使用する遺伝子のゲノムへの挿入がないことの保証など安全面において，ES 細胞とは違った課題があるなど，まだ難しい点が残されている。しかし日本では臨床研究がまもなく始まろうとしており，課題解決に向けた取り組みが加速しており，今後大きな期待が寄せられている。

iPS 細胞の登場は，医療分野にさらなる波及効果も生み出している。再生医療のソースに限れば，iPS 細胞作製によるリプログラミング技術は特定の分化能を有する体性幹細胞への形質変換（ダイレクトリプログラミング）（図 1）も期待できることを示しており，実際複数の遺伝子導入で心筋細胞[4]や軟骨細胞[5,6]が得られたとの報告がされた。また疾患患者由来細胞を用いた発症機構の解明や創薬スクリーニングなどへの利用にも期待が高い。

Ⅴ 細胞から器官へ～三次元培養・組織工学との融合による再生医療

再生医療研究の進展と呼応するように，周辺技術の開発が進められている。現在の幹細胞移植においては，局所的な細胞の回復は望めても臓器・器官を完全に補完することまでには至っていない。そこで考えられているのが，三次元培養による器官構築に向けた取り組みと，人工臓器と再生医療を組合せたハイブリット治療の取り組みであろう。

三次元培養は，細胞をシート状にしたうえで積層化したり[7]，コラーゲンなどの細胞外マトリックスを利用した足場材料に細胞を播種したりすることなど，さまざまなことが行われている。特に最近注目されている 1 つが，脱細胞化技術を利用した方法であろう。幹細胞を移植する際，特に肝臓や腎臓などは三次元構造の構築が難しいとされている。そこで生体の組織構造をそのまま残したまま器官内にある細胞のみを除き（脱細胞化），そこに新たな細胞を生着させる方法が試みられている。この脱細胞化技術を使って肝細胞機能を体外で維持させることに成功したとの報告もでてきた[8]。さらにイメージング技術と 3D プリンターによる生体組織を模した足場材料を作製する試み[9]もあり，これは機能欠損部位に対してオーダーメード的に補完・代替できる可能性をもち，最新の IT 技術や工学技術との組合せによって患者の QOL をも考慮した治療に向けて今後の展開が注目される。

一方，人工臓器の開発は，著しく機能低下した腎臓や心臓などの代替として今や医療において欠かすことができない。しかし実際には完全な機能回復までには至らず，一度人工臓器をつけたらなかなかそこから離脱することは難しいとされており，機器の管理や医療経済の面から課題も多い。そこで，一時的に機能低下した臓器を人工臓器に

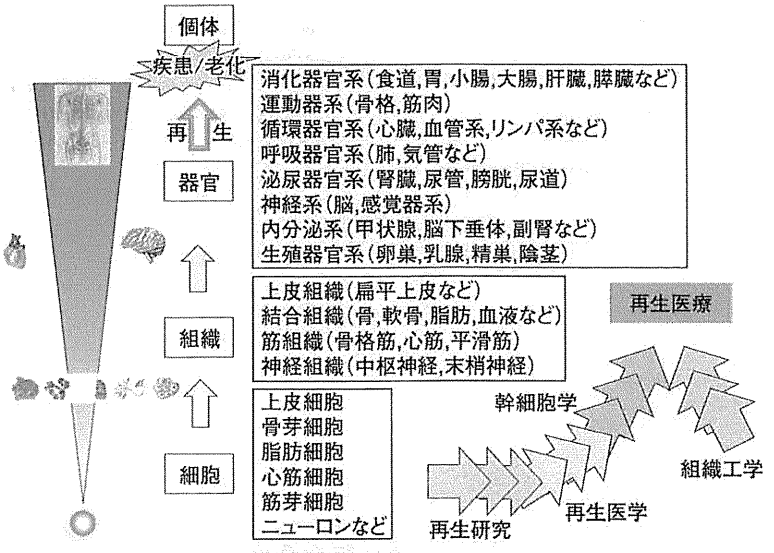

図3　再生医療は細胞再生から組織・器官再生へ

より補完しつつ，幹細胞移植により機能再生を図り機器からの離脱を図る試みが始まろうとしている。幹細胞の移植による有効性には移植した細胞がいかに臓器に生着するかが課題であるが，そのための環境を人工臓器による補助によって作り出せる可能性があり，今後の展開が楽しみである。

おわりに

これまでみてきたように，幹細胞移植による再生医療に活かせる細胞ソースとして間葉系幹細胞，脂肪幹細胞，ES細胞，iPS細胞などさまざまな幹細胞がある。さらにはより発生初期段階に近いとされるヒト基底状態ナイーブな多能性幹細胞[10]やクローン技術を用いた核移植ES（ntES）細胞[11]，また癌幹細胞といった新たな幹細胞も登場してきており，今後も見出されてくる可能性もある。将来的には，各人の疾患部位や症状などによってどの幹細胞がベストであるか選択できるようになるかもしれない。さらに再生医療実現化に向けた技術開発の進展によって再生医療との組合せによる医療も期待できる。ヒトの体を構成する基本単位である細胞を用いる再生医療は，まさにこれまで難しかった多くの疾患に対する細胞レベルでの補修を可能とし，さらには臓器・器官の修復まで行おうとするところまで来ている（図3）といえるであろう。

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