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FORUM REVIEW ARTICLE

Transcriptome Analysis in Endothelial Progenitor Cell Biology

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Abstract

The use of endothelial progenitor cells (EPCs) is a promising new treatment option for cardiovascular diseases. Many of the underlying mechanisms that result in an improvement of endothelial function *in vivo* remain poorly elucidated to this date, however. We summarize the current positions and potential applications of gene-expression profiling in the field of EPC biology. Based on our own and published gene-expression data, we demonstrate that gene-expression profiling can efficiently be used to characterize different EPC types. Furthermore, we highlight the potential of gene-expression profiling for the analysis of changes that EPCs undergo during culture and examine changes in gene transcription in diseased patients. Transcriptome profiling is a powerful tool for the characterization and functional analysis of EPCs in health and disease. *Antioxid. Redox Signal.* 15, 1029–1042.

Introduction

Cardiovascular diseases are the leading cause of morbidity and mortality in the Western world and impose an ever-increasing socioeconomic burden (78, 140). The underlying pathological mechanism of these diseases is atherosclerosis, a multifactorial chronic inflammatory disorder characterized by progressive endothelial dysfunction and formation of lipid-rich plaques containing macrophages and smooth muscle cells within the vascular wall (70). Current therapies consist of slowing the progression of atherosclerosis through risk-factor reduction by changes in lifestyle and pharmaceutical intervention. When focal plaques occlude major arteries, circulation can be restored through surgical or endovascular interventions (86). These therapies have limitations, however, and many patients reach a point at which treatment options are exhausted (43, 86). Through the rapidly developing field of regenerative medicine, a third treatment modality may be available in the form of progenitor cell therapy. This therapy aims at restoring tissue perfusion and preserving end-organ function by using progenitor cells to improve arterial dysfunction or to stimulate neovascularization.

The concept of regenerative medicine in cardiovascular disease dates back to a study by Asahara *et al*. in 1997 (5). The authors demonstrated that bone-marrow (BM)-derived cells with properties resembling primitive vascular cells could be isolated from peripheral blood and that these cells contribute to the formation of new vessels (4, 6). The cells were termed endothelial progenitor cells (EPCs), and the following decade saw a large number of publications confirming and extending the findings of Asahara *et al*. Later, it was shown that numbers of EPCs are reduced in the blood of patients with arterial disease or diabetes mellitus (DM) (21, 30–32, 45, 48, 66, 75, 83, 119, 129, 135) and that circulating EPC numbers can serve as a biomarker to predict future cardiovascular events (108, 135). These observations suggest a role for EPCs in vascular homeostasis (15) and the potential use of progenitor cells as a treatment for cardiovascular disease. Several therapies have been devised in the form of autologous transplantation of BM (47, 114, 118) or mobilized progenitor cells (49, 51, 58), or alternatively, by increasing progenitor cell numbers and function in the circulation through the administration of growth factors (3, 29, 81, 127).

However, much debate exists about which cell types actually contribute to the functional improvement and increased perfusion observed in clinical trials thus far. Until recently, the term EPC was used rather promiscuously, referring to circulating and cultured cells alike, although different phenotypes arise when different isolation techniques and culturing methods are applied (55, 57, 93, 120, 142). Furthermore, seemingly minor differences in experimental procedures produce widely differing results (23, 42, 109). Therefore, principal questions remain unanswered: What exactly constitutes *the* EPC? From which lineage do EPCs originate? Are cultured EPCs analogous to their counterparts *in vivo*? Until now, EPC biology has been very much an empiric science, in which several therapies have been tried *in vivo* and even in a clinical setting, without necessarily a complete or accurate understanding of mechanistic underpinnings. A more integrated view of EPC biology may be necessary to

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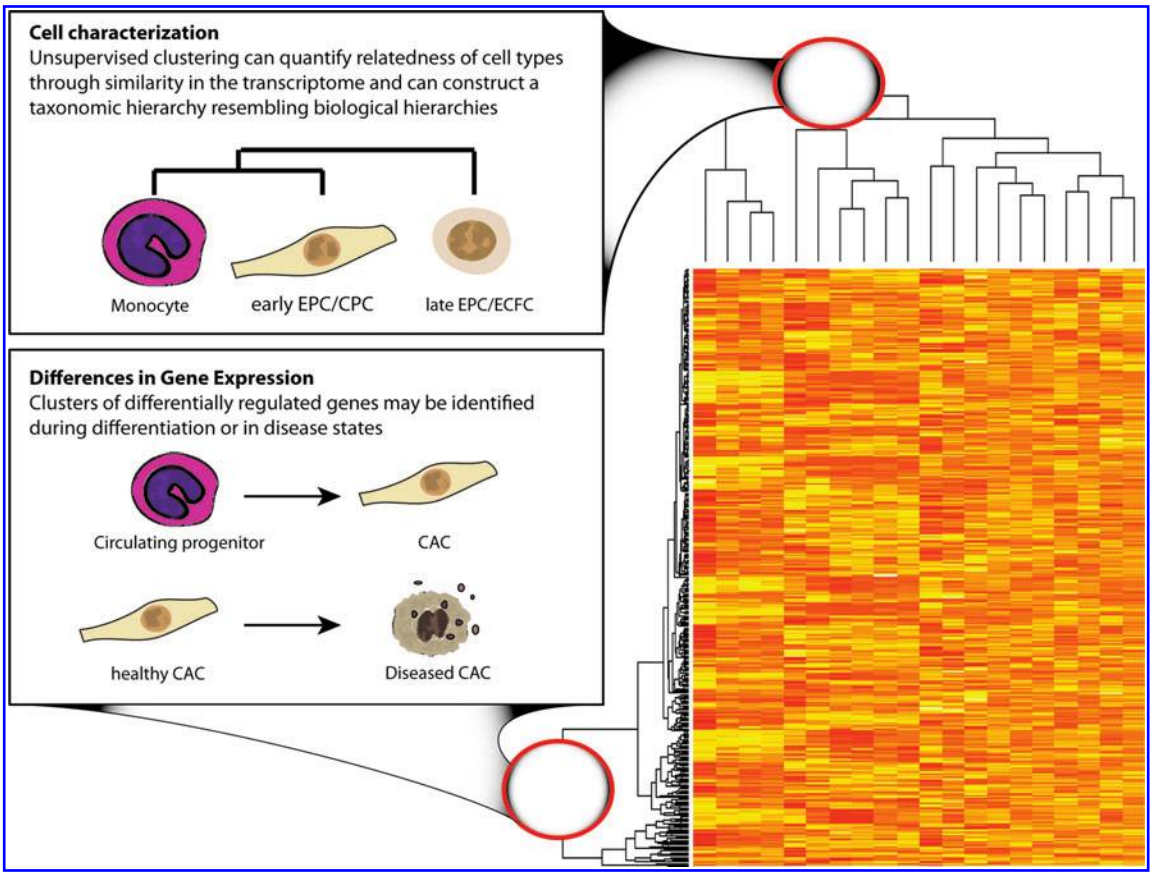

FIG. 1. Potential applications of gene expression profiling in EPC biology. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

reconcile findings and to develop promising strategies for forthcoming therapies. Other fields, such as oncology, have benefited greatly from gene-expression profiling to identify cellular markers and targets for treatment. Here we review the implementation of transcriptional profiling in the field of EPC biology.

Transcriptome Analysis

The Human Genome Project (68, 130) and the subsequent development of technologies that enable large-scale genomic analyses (26a, 36, 72) have widened the scope of molecular biologic analyses to an unprecedented degree. At present, a whole range of "-omics" has been developed, each dedicated to giving a comprehensive view of one aspect of cellular functioning (104, 105). Among the "-omics", transcriptomics, or gene-expression profiling, is currently the most widely implemented. As methods and guidelines for reporting experiments have been largely standardized (11), reproducibility has reached a level at which direct comparisons of multiple experiments have become possible (76).

The most common method for transcriptional profiling is the hybridization of target nucleotides to oligonucleotide DNA microarrays. These arrays consist of a large number of gene-specific short nucleotide sequences, called probes, immobilized as millions of identical copies at a unique location on a solid support (54, 72, 73). Usually, each gene is represented by one or more probes, and each probe is present in duplicate or multiplicate on the microarray. Labeled target nucleotide sequences, derived from mRNA, are hybridized to their complementary probes to give a quantitative estimate of the abundance of the target mRNA sequence. As microarrays are constructed to probe the transcriptome as comprehensively as possible, it is not necessary to know beforehand which genes are involved in the process under investigation (73). This makes gene-expression profiling particularly suited to generate hypotheses about processes of which underlying mechanisms are poorly understood. In addition, it makes analyses relatively unbiased toward expected or hypothesized effects.

Transcriptome analysis using microarrays has been used to answer questions that are not unlike the questions that currently dominate the field of EPC biology. Gene-expression profiling may provide insight into the characterization of EPCs, alterations of the EPC phenotype during differentiation, and comparison of EPCs in healthy and diseased states (Fig. 1). One potential application of gene-expression profiling is the characterization of closely related cell types. Microarrays have, for instance, been extensively used to distinguish different tumor phenotypes among morphologically very similar tumors (2, 40, 98, 133, 139). The advantage over methods investigating only a few markers, such as flow cytometry, immunohistochemistry or polymerase chain reaction (PCR), lies not only in number of markers investigated. As microarrays probe the transcriptome comprehensively, transcriptional profiling allows the attachment of a meaningful quantitative measure to the relatedness of different transcriptomes (59, 63). This allows the construction of a hierarchy of relatedness through clustering algorithms (63) that

Table 1. Overview of Published EPC Transcriptome Data

First author	*Year*	*EPC type*	*Comparison*	*Microarray platform*
Dernbach, E. (24)	2004	CACs	CACs vs. $CD14^+$ monocytes, HMECs, and HUVECs	–
Urbich, C.(122)	2005	CACs	CACs vs. $CD14^+$ monocytes, HMECs, and HUVECs	HG-U95Av2
Urbich, C.(124)	2005	CACs	CACs vs. $CD14^+$ monocytes, HMECs, and HUVECs	HG-U95Av2
Fiorito, C.(33)	2008	CACs	Effects of vitamin C and E on resting and TNF-α–treated CACs	HG-U133 Plus 2.0
Igreja, C. (54)	2008	CACs	CACs at day 1 and day 13 in culture	HG-U133A
Desai, A. (25)	2009	CFU-Hills	Changes in gene expression after 3 months of exercise; comparison of CFU-Hill with other cell types in database	HG-U133 Plus 2.0
Maeng, Y.S.	2009	CB-CACs, CB-ECFCs	Differences in gene expression between the two progenitor types	HG-U133 Plus 2.0
V Oostrom, O. (127)	2009	CACs	Differences in gene expression between patients with DM I and healthy controls	Illu Human Ref-8
Medina, R.J. (84)	2010	CACs, ECFCs	Comparison between CACs and ECFCs, as well as HDMEC and $CD14^+$ monocytes	Illu WG-6 v3.0
Zeisberger, S.M. (144)	2010	ECFCs	Gene expression in EGM-2 medium with FCS vs. SF-1	HG-U133 Plus 2.0
Tan, K (118)	2010	ECFCs	Cells from patients with proliferative diabetic retinopathy vs. healthy controls	HG-U133 Plus 2.0

may reflect the taxonomy in biologic hierarchies, such as the hematopoietic hierarchy (13, 132). Cells of uncertain ontogeny may be compared in this fashion with existing hierarchies to attain a better understanding about their origins. Ideally, BM-resident and circulating EPCs could be integrated into an expression profile–based hierarchy ranging from the hemangioblast to the terminally differentiated endothelial cell. Moreover, transcriptomes of different cultured EPC phenotypes could be compared with the endothelial and the hematopoietic hierarchy for a proper transcriptome-based classification of these cells (54). The changes that isolated EPCs undergo during the selection and differentiation process of culture can be elucidated, as it seems that isolation and culture alter the characteristics of a cell type to some degree, allowing cells both to acquire markers and perhaps to induce functional alterations (94, 120).

One of the pillars of regenerative cellular therapies is the use of autologous cells in the treatment of disease. In the case of EPCs, however, the function of these progenitor cells may be impaired in diseased subjects (45, 119, 128). Identifying which pathways and mechanisms are affected by disease processes may provide leads for interventions or adaptation of culture protocols that improve progenitor cell function. Taken together, transcriptome analysis has the potential to answer principal questions and to enhance our understanding of EPC identity, differentiation, and function in health and disease. In this review, we address these issues and provide an overview of published EPC transcriptome data (Table 1).

EPC Identity

During embryonic development, endothelial cells and hematopoietic cells are thought to arise from a common progenitor, termed the hemangioblast (99, 100), although the exact sequence of intermediates is still unknown (67) (Fig. 2). On endothelial differentiation, the newly formed angioblasts acquire a set of early endothelial markers, including vascular endothelial growth factor receptor 2 (VEGFR-2/KDR), CD31, and TEK tyrosine kinase, forming primitive blood islands (15). Vascular networks sprout from these primitive blood islands in a process termed vasculogenesis. These networks extend, providing an integrated vasculature throughout the body, in a process called angiogenesis. Until the 1990s, the prevailing paradigm was that, although endothelium has the capacity to proliferate locally (37) in the adult, as was demonstrated in grafts (35, 79, 92) or in transplanted organs (62), or even by shedding circulating endothelial cells (115), no vasculogenesis occurs in adult life. In 1997, Asahara *et al*. (5), however, demonstrated the existence of circulating BM-derived cells that are able to form colonies that have a high resemblance to primitive vascular structures, *in vitro*. These cells had characteristics of endothelial cells, such as CD31, KDR, and endothelial nitric oxide synthase (eNOS) expression, but also displayed markers associated with early progenitor cells, such as CD34 (5, 120) and CD133 (89), and were consequently termed EPCs. It was demonstrated that these EPCs also integrate into damaged vessels in a hindlimb-ischemia model (4, 5, 116). Kalka *et al*. (61) demonstrated that these cells could have therapeutic applications by injecting human EPCs into an athymic nude mouse hindlimb-ischemia model. Mice that were injected with EPCs showed increased restoration of blood flow compared with untreated controls or mice treated with mature endothelial cells (61). A translation toward a clinically viable therapy was devised shortly thereafter, by using local infusion of autologous BM-derived cells in patients with coronary or peripheral arterial disease (9, 47, 114, 118). Further evidence for the importance of EPCs in cardiovascular disease came from a number of studies investigating the number of EPCs in patients with arterial disease.

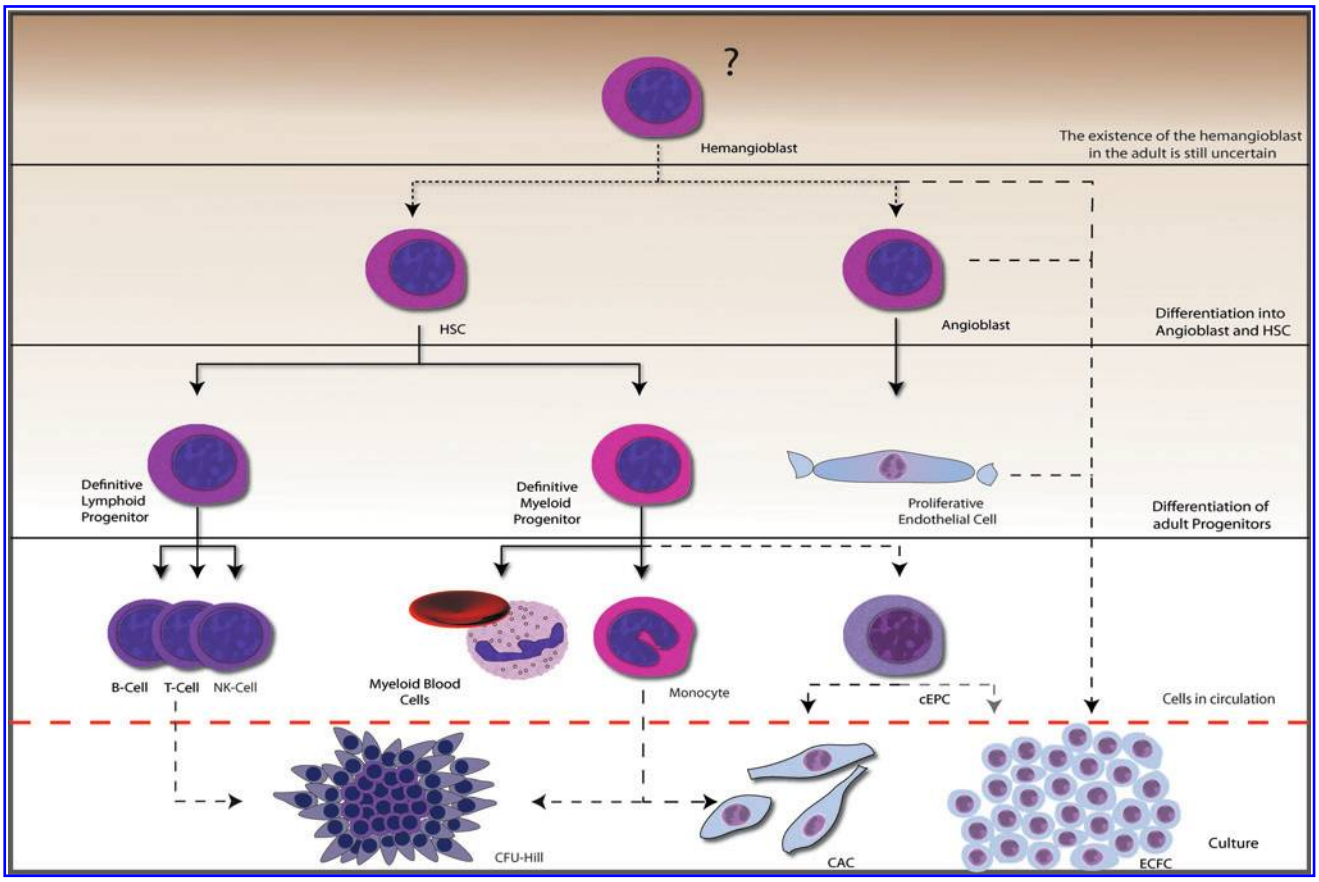

FIG. 2. Putative origin of different EPC types observed in circulation and in culture. The exact sequence of differentiation steps from the hemangioblast to the hematopoietic and endothelial lineages still remains a topic of intense investigation. CACs and CFU-Hills are thought to arise from myeloid leukocytes, whereas ECFCs are true endothelial cells. *Solid lines* indicate well-established routes of differentiation; *dashed lines*, current uncertainties. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

The amount of both circulating EPCs (cEPCs, defined as $CD34^+$, KDR^+ cells) and EPCs arising in culture was found to be reduced in patients with coronary artery disease, as was the migratory capacity of the EPCs cultured from these patients (128). A study by Hill *et al*. (48) introduced a new culture assay using peripheral blood mononuclear cells (PBMCs) that involved a preplating step to remove circulating differentiated endothelial cells, as endothelial shedding in cardiovascular disease was considered a possible "contamination" in EPC culture. The authors then counted the colonies, now commonly called CFU-Hills, which arose in the second plating step. The number of colonies was found to be inversely related to the Framingham cardiovascular risk score and robustly related to endothelial function, as measured with flow-mediated brachial dilatation.

Evidence began to accumulate that a large heterogeneity exists in cultured EPC phenotypes. In 2000, Lin *et al*. (71) had shown that another, distinct phenotype of EPCs, which is highly proliferative and closely resembles endothelium, arises in long-term culture. This contrasted with the previously studied spindle-shaped EPCs that formed after culturing PBMCs for 7 days, including a washing step at day 4 to remove nonadhering cells (128, 129). Hur *et al*. (55) closely characterized the two phenotypes that arise after short- and long-term culture and coined the terms early- and late-outgrowth EPCs. Later these cells were renamed circulating angiogenic cell (CAC) and endothelial colony-forming cells (ECFCs), respectively, although at present, no guidelines exist for a consistent nomenclature of EPC subtypes. It was discovered that CACs have little or no proliferative capacity (55, 146) and are unable to form tubular networks on basement membrane matrix (Matrigel) (85). Interestingly, they do colocalize to nodes in vascular networks (55) formed by endothelial cell lines *in vitro*, but do not fully integrate into the networks (110). CACs do secrete large amounts of growth factors (55, 110) and likely participate in extracellular matrix (ECM) remodeling (41), thereby promoting angiogenesis in a paracrine fashion (110, 143). Furthermore, it was shown that CACs express myeloid lineage markers, such as CD14 and CD45 (33, 122) and exhibit phagocytic ability (96, 146). ECFCs, conversely, are characterized by the absence of myeloid markers and appear more closely to resemble endothelial cells. For instance, they form tubular structures when plated on Matrigel and form cobblestone-shaped monolayers with smooth cytoplasm in culture (55, 71, 142). Also, they have been shown to be highly expandable (38) and do not incur genomic aberrations during expansion (97). The colony-forming assay introduced by Hill *et al*. (48, 135) seemed to produce a third, mixed, EPC phenotype, as the colonies were shown to consist of both monocytes and lymphocytes (93, 101, 124).

How the cultured phenotypes relate to cEPCs depends on how the latter are defined. A common definition of cEPCs is

Table 2. EPC Glossary

Name	*cEPC*	*CAC*	*CFU-Hill*	*ECFC*
Synonyms	EPC, circulating EPC	EPC, early outgrowth, CPC, EPC, CE-EPCs, CMMCs, ELC, PAC, APC, EAC	EPC, CFU	EPC, late-outgrowth EPCs, ECs, BOECs, OECs, EPDCs
Definition	By flow cytometry, usually as $CD34^+/KDR^+$ or $CD34^+/KDR^+/CD133^+$	Cells derived after plating of PBMCs for 4–7 days on fibronectin-coated wells, in EGM-2 medium. Bind lectin and take up LDL	Cells in colonies generated from re-plated PBMCs in the Hill assay	Cells derived from late-appearing colonies with a cobblestone appearance
Origin	Depends on exact marker definition	Myeloid, closely related to monocytes	Mixture of T cells and monocytes	Unclear, appears not be derived from hematopoietic line

APC, angiogenic progenitor cell; BOECs, blood-outgrowth endothelial cells; CAC, circulating angiogenic cell; CD34, cluster of differentiation 34; CE-EPCs, culture-expanded EPCs; CFU-Hill, colony-forming unit, Hill-type; CMMCs: culture-modified monocytic cells; CPC, circulating progenitor cell; EAC, early outgrowth angiogenic cell; ECs, endothelial cells; ELC, endothelial-like cell; EPC, endothelial progenitor cell; EPDCs, endothelial progenitor–derived cells; KDR, kinase insert domain receptor (VEGFR2); OECs, outgrowth endothelial cells; PACs, proangiogenic cells.

based on the coexpression of CD34 and KDR; this definition includes mostly cells from the hematopoietic lineage (17, 69, 93, 120) that comprise a subset of the cells that will form CACs in culture (103).

For clarity, we adopt the following nomenclature for the various circulating and cultured progenitor cells (Table 2). We refer to circulating EPCs, as detected by flow cytometry, as cEPCs; monocytic early outgrowth cells are denoted as circulating angiogenic cells (CACs); colonies obtained with the Hill assay will be called CFU-Hills; and late-outgrowth endothelial cells will be called endothelial colony-forming cells (ECFCs). The term EPCs will be used to describe relevant progenitor cells in general.

Definition and characterization of progenitor cells is historically based first and foremost on cell-surface antigens associated with either or both endothelial cells and progenitor cells, which can be detected by using flow cytometry and immunohistochemistry (120). The antigens selected to define EPCs are selected on the basis of previously established associations with either stem cell characteristics, as, for instance, CD34 (5) and CD133 (89), or endothelial cells, such as KDR/VEGFR2, eNOS, or von Willebrand factor (vWF) (5). Although this reasoning has proven very effective in advancing the field of regenerative medicine, characterization by flow cytometry is not very specific, as much overlap occurs in markers between cells in the hematopoietic line, especially in early progenitor cells (39, 93). Furthermore, this approach is prone to errors with regard to cultured cells, as markers may be lost (89) or acquired (20, 94, 107, 120) during mononuclear cell isolation and culture (33). The introduction of a classification on the basis of functional capabilities that are relevant for cell therapy, such as the secretion of growth factors (110), the ability to proliferate (57, 142) or to form tubular structures on Matrigel, has been a leap forward in creating a conceptual framework to understand the roles of circulating and cultured EPCs in the restoration of blood flow to ischemic tissues (141, 142).

Starting from a cell population that displays certain phenotypic or functional characteristics, transcriptional profiling may be used rationally to identify unique markers or genes associated with a particular cell-type function.

Transcriptional profiling provides a characterization method that is relatively unbiased because it indiscriminately includes the entire set of transcribed genes. In addition, the existence of online repositories for gene-expression profiles allows comparisons to be made between cell types of different origins. To illustrate how gene-expression profiles may aid in the classification of EPCs, we conducted an analysis of publicly available transcriptomes from different cell types and tissues of mesodermal origin (see Supplementary Data; Supplementary Data are available online at www.liebertonline.com/ars). With this approach, at least one transcriptome of CACs, ECFCs, and CFU-Hill each on the Affymetrix HG-U133 Plus 2.0 array could be identified. In addition, we included a further 45 transcriptomes from 30 studies, representing various cell types from hematopoietic and mesenchymal tissues. Results of the hierarchic clustering of all transcriptomes show a clear distinction between hematopoietic and other mesenchymal lineages (Fig. 3). The cord-blood–derived ECFCs cluster with the endothelial cell lines included, showing high similarity to human umbilical vein endothelial cells (HUVECs) and human microvascular endothelial cells (HMECs). Interestingly, the cord-blood–derived CACs cluster with the hematopoietic cells and show greater similarity to adult $CD14^+$ monocytes than to $CD34^+$ cells from cord blood. The correlation between CACs and monocytes was not as high as correlations between samples of similar origin, making the exact position of CACs in this hierarchy less clear cut. The CFU-Hills seem to cluster loosely with $CD34^+$ cells and were previously found more closely to resemble T cells (26). Possibly the ambiguous clustering reflects the fact that CFU-Hill colonies consist of both myeloid and lymphoid cells (101).

These results demonstrate that it is possible to use transcriptome analysis to distinguish between different cell types and to place cell types of unclear or disputed origin in an established biologic context. An important caveat when comparing transcriptome data from different sources is that culture conditions may influence gene expression, resulting

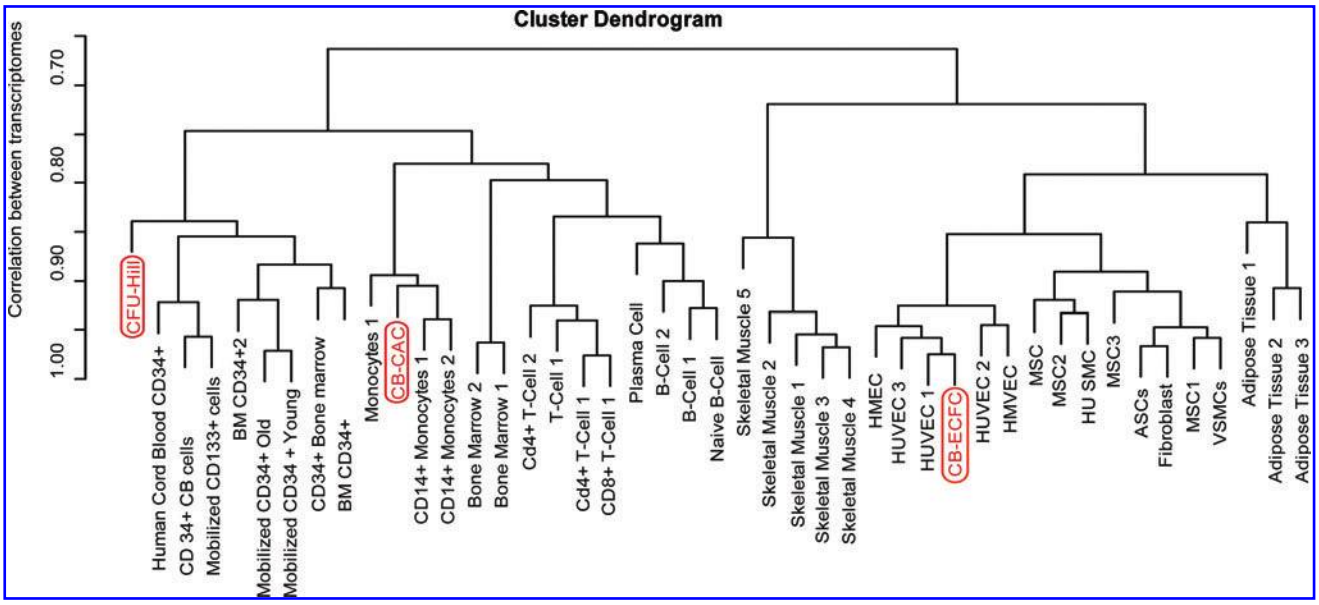

FIG. 3. Dendrogram generated by unsupervised hierarchic clustering of transcriptomes of various mesenchymal tissues. The Y-axis represents similarity based on correlation (Pearson's *R*). ASC, adipocyte stem cell; HM(V)EC, human microvascular endothelial cell; HUSMC, human umbilical cord smooth muscle cell; HUVEC, human umbilical vein endothelial cell; MSC, mesenchymal stromal cell. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

in altered gene-expression profiles. The transcriptome data used in this study are derived from primary blood or tissue samples, as well as cell lines cultured in different media. Despite the varying conditions, the clustering correctly identified the interrelatedness of the included expression profiles. Furthermore, it is underlined that CACs are myeloid cells, showing similarity to $CD14^+$ monocytes, whereas ECFCs show a high similarity to endothelial cells.

Similar results are described by Medina *et al*. (82), who directly compared the transcriptomes of CACs and ECFCs from single donors with microvascular endothelial cells and $CD14^+$ monocytes. Clustering analysis showed a pattern similar to our analysis, with CACs more closely resembling monocytes and ECFCs closely resembling microvascular endothelial cells. Genes involved in hematopoietic development, such as *RUNX1*, *WAS*, and *LYN*, and genes involved in the immune response, including TLRs, CD14, and HLAs, were significantly more highly expressed in CACs. In contrast, genes involved in angiogenesis, such as Tie2, eNOS and Ephrins were highly expressed in ECFCs. In addition to transcriptional profiling, Medina *et al*. also compared the proteomes of the different cell types and were able to identify several unique proteins in CACs and ECFCs. The authors have yet to characterize the spots with mass spectrometry to identify these potential marker proteins for the different progenitor cells.

The described microarray data should be interpreted in conjunction with those from other recent studies. Piaggio *et al*. (91) and Otten *et al*. (87) investigated progenitor cells in patients diagnosed with chronic myeloid leukemia (CML). This type of leukemia is characterized by a supernumerary chromosome arising from translocation of the *BRC* and *ABL* genes on chromosome 9 and 22, respectively. The aberrant chromosome, called the Philadelphia chromosome, can be detected with fluorescent *in situ* hybridization (FISH) karyotyping or PCR. Piaggio *et al*. (91) showed that a significant portion of CACs, but none of the ECFCs, of patients with CML showed the translocation. As CML tumor cells arise from an early progenitor in the myeloid lineage, this study confirms that CACs are myeloid/monocytic cells and that ECFCs do not arise from CACs directly, but from a distinct endothelial lineage.

An explanation for the fact that CACs show a large number of endothelial surface markers is provided by Prokopi *et al*. (94). The authors used proteomics to identify a large number of surface markers on CACs and compared these with genes expressed in these cells. It was shown that many of the endothelial surface markers are not expressed but are acquired from platelet microparticles during isolation and culture. These findings indicate that the different EPC phenotypes that arise in culture not only are functionally different, but also have differing expression profiles and arise from distinct lineages, which was confirmed by the comparison of transcriptome profiles.

Changes in Gene Expression during EPC Culture

Ex vivo culture and expansion of progenitor cells is one of the most important strategies to overcome a low number of EPCs and to develop future regenerative therapies. Moreover, an interim culturing step provides a window for interventions, as culture conditions can be designed to overcome functional deficits that progenitor cells from patients may have. Transcriptomics provides a method of systematically comparing changes that cells undergo in culture, and of identifying genes that may be important for optimizing cell therapy.

The majority of preclinical studies investigating therapeutic applications of progenitor cells have hitherto been conducted with CACs (61, 122), perhaps because, in contrast to ECFCs, CACs are relatively easily and reproducibly isolated. It is interesting to note that although many studies have shown that CACs are able to restore perfusion *in vivo* (16, 18, 122, 143), $CD14^+$ monocytes, from which CACs predominantly arise

(33, 65), do not show this effect (14, 122). This suggests that an active subset of $CD14^+$ monocytes (27) is selectively enriched during attachment in CAC culture (102, 122), or that differentiation activates proangiogenic mechanisms in CACs. Only a handful of studies have investigated transcriptional changes that occur during selection and differentiation from PBMCs or $CD14^+$ monocytes to CACs. Dernbach *et al*. (25) were the first to describe differences in the transcriptome between CACs, monocytes, and differentiated endothelial cells (HUVECs and HMECs). The authors show that a number of antioxidant genes are selectively upregulated in CACs, in particular, manganese superoxide dismutase (MnSOD), glutathione peroxidase (GPX), and catalase (Cat). These genes confer a high resistance to hydrogen peroxide–induced oxidative stress, as was demonstrated by a fluorescent oxidation-sensitive dye. It was demonstrated that CACs are less likely than endothelial cells or monocytes to go into apoptosis in the presence of high concentrations of reactive oxygen species (ROS) or during serum starvation, a condition associated with increased oxidative stress.

Finally, it was shown that the antioxidant capacity of CACs is dependent on MnSOD, GPX, and Cat by using knockdown or specific inhibitors against these antioxidant enzymes. To reach intracellular ROS levels similar to those in HUVECs, inhibition of all three proteins was necessary, showing the redundancy of the antioxidant system in CACs. Interestingly, the authors also showed that CAC migration is inhibited by elevated levels of ROS, and that migration is further reduced by inhibition of antioxidant genes. A study by Urbich *et al*. (123) also compared expression profiles of $CD14^+$ monocytes, CACs, and HUVECs. The authors identify a number of genes that are upregulated in CACs, when compared with HUVECs and monocytes, most notably, a cluster of proteases including cathepsins H, L, and O. The involvement of cathepsin L in ECM remodeling was subsequently demonstrated, as was the requirement of cathepsin L for improvement of neovascularization in a hindlimb-ischemia model after CAC infusion. In a second article, the same group described that a large number of growth factors and chemokines, such as VEGF-A, interleukin (IL)1, IL-2, IL-8, and fibroblast growth factor (FGF)-A, are highly expressed in both monocytes and CACs when compared with endothelial cells (121). During the transition from monocytes to CACs, a second cluster of growth factors was increasingly expressed, including VEGF-B, platelet-derived growth factor (PDGF) A, stromal cell–derived factor (SDF)-1α, insulin-like growth factor (IGF)-1, and hepatocyte growth factor (HGF). These growth factors were shown to increase greatly the migratory capacity of HUVECs, suggesting that CACs exert paracrine effects on endothelium.

Collectively, these studies effectively used gene-expression profiling as a screening method to identify important target genes or gene clusters for the function of CACs. Other authors attempted to provide a more-comprehensive overview of transcriptional changes during selection and differentiation. Igreja *et al*. (56) examined the expression profiles of adherent human cord-blood–derived $CD133^+$, KDR^+ or $CD34^+$, KDR^+ cells at day 0 and day 13 of plating the cells in culture. The authors then identified highly expressed genes and divided them into three categories: genes present at day 0 and absent at day 13, genes present at day 13 and absent at day 0, and genes showing a high expression level at both time points. Gene ontologic analysis subsequently showed enrichment in membrane proteins, G protein–coupled receptors, nuclear proteins, and genes involved in cholesterol biosynthesis at day 13. Several well-known markers for primitive endothelial progenitors were found to be highly expressed. KDR, angiopoietin 1, hypoxia-inducible factor (*HIF*)-1α, heme oxygenase (*HO-1*), *HOXA5*, and Delta-like ligand 4 (*Dll4*) were found to be highly expressed throughout differentiation; expression of TEK could be detected only on day 0, whereas Flt1 could be detected only on day 13. Furthermore, the expression of two genes associated with homing of progenitor cells, SDF1α and the IGF2 receptor, was increased on day 13, although CXCR4 expression decreased during differentiation. The authors also noted that cell-adhesion molecules, such as L-selectin, integrins α_4, α_5, α_V, and ß$_2$ are highly expressed throughout the differentiation process. The authors further report the emergence of endothelial colonies at certain points in the study, but it is unclear how this affects the results of the gene-expression analysis.

The study by Medina *et al*. (82), mentioned earlier in this article, includes samples of both monocytes and CACs. As it was the primary aim of the authors to demonstrate the difference between ECFCs and CACs, however, differences in transcription between $CD14^+$ monocytes and CACs were not investigated in detail. Furthermore, only a single transcriptome of $CD14^+$ cells is included, which does not allow the identification of significantly upregulated genes. To present a more reliable overview of changes in gene expression that CACs undergo in culture, we included two unpublished microarray expression profiles from our laboratory, obtained with pooled mRNA samples from PBMCs and CACs of 10 healthy human volunteers. Gene-expression profiles of monocytes/PBMCs and CACs were compared by using the rank-product method (12). This nonparametric method ranks genes according to the difference in expression (fold change) between experimental conditions and subsequently identifies genes that are consistently up- or down-regulated (12). The advantage of this method is that it makes minimal assumptions about the distribution of the data and is therefore very robust, especially in the analysis of small datasets. In addition, it allows identification of differentially expressed genes in a meta-analysis across multiple small datasets (50). In total, 312 genes were found to be differentially expressed in CACs (Supplementary Data). Functional classification by using DAVID (24) revealed activation of distinct functional gene clusters after selection and differentiation (Table 3). The most striking upregulation was observed in genes regulating the uptake and metabolism of lipids. Apolipoproteins and scavenger receptors were found to be highly expressed in CACs but not in monocytes. The exact significance of this finding is unclear, although it is not quite unexpected, given that one of the characteristic features to define CACs is the uptake of acetylated low-density lipoprotein (LDL) as well as the well-known association of genes involved in cholesterol metabolism with progenitor cells (88). In accordance with the findings of the previously mentioned studies (121, 123) a number of ECM remodeling proteases from the cathepsin and matrix metalloproteinase families were also more highly expressed, as were SDF1α, VEGF-B, and a number of antioxidant enzymes, especially genes involved in intracellular thiol redox control. These studies show that CACs consistently upregulate a series of genes when placed in culture. Many of the upregulated genes have been

Table 3. Genes Upregulated in CAC Compared with MNCs/Monocytes

Gene function	*Selection of upregulated genes in CAC*
Lipid Metabolism	*ABCA1, ACAT2, AKR1B1, ALDH1A1, ALDH4A1, ALDH7A1, APOC1, APOC2, APOE, DHRS9, FABP3, FABP4, FABP5, FABP5L1, GLA, LIPA, LPL, MGLL, MSR1, NR1H3, OLR1, PLTP, PPAP2B, PPARG3, PTGR1, SCD*
ECM remodeling/ homing	*CTSB, CTSD, CTSK, CTSL1, LGMN, MMP12, MMP7, MMP9, TIMP2, LAMB2, LAMC1, ITGB5*
Oxidative stress	*NQO-1, ATOX1, GCLC, GPX3, MT1G, MT2A, PRDX1, TXN,TXNRD1*
Chemotaxis	*CXCL16, CXCL12 (SDF1α), CCL8 (MCP-2), VEGFB, TNFRSF12A, IGF1*

shown to be important for the function of CACs, especially genes involved in homing and ECM remodeling (7, 19, 41, 46, 53, 90, 123, 131, 145). These changes in gene expression may explain the functional differences between $CD14^+$ monocytes and CACs. At this point, however, it is unclear whether these changes occur because of a differentiation mechanism or the selection of an active subset of monocytes in culture. Very little is known about the differentiation of ECFCs in culture, as the circulating progenitor cell from which ECFC colonies develop has not yet been identified. Current evidence indicates that ECFCs arise from a very small fraction of $CD34^+$, KDR^+, $CD133^-$, $CD45^-$ cells (17, 120). The estimated prevalence of ECFCs is less than one per 1 million nucleated cells (57, 97), however, which poses difficulties for the characterization of ECFCs. A recent study has attempted to use gene-expression profiling to identify genes that are important for the outgrowth of ECFCs (144). The authors compared different culturing media, with the intention of identifying an animal-serum–free equivalent to the conventional medium, with fetal calf serum. No outgrowths occurred in serum-free media, however, and replated ECFCs showed greatly reduced proliferation. Gene-expression analysis showed significant upregulation in genes related to lipid biosynthesis, stress response, and apoptosis, and a marked downregulation in genes associated with proliferation. The authors concluded that lipid supplementation may enhance ECFC outgrowth in serum-free media. This study illustrates how transcriptome analysis can be used to find leads for improving culturing methods in the development of a consistent cell therapy.

EPCs in Health and Disease

Next to providing insight into EPC identity and differentiation, transcriptome analysis provides a powerful tool to elucidate molecular changes in EPCs of patients with (risk factors for) cardiovascular disease. Several studies have shown that numbers of cEPCs (128), cultured CACs (128), and CFU-Hills (48) are reduced in patients with arterial disease or risk factors for cardiovascular disease, such as metabolic syndrome (138), chronic kidney disease (60, 137), and diabetes (119). Furthermore, it has been shown that BM from patients with ischemic cardiomyopathy (45) has an impaired regenerative potential when compared with BM from healthy donors. A role for disease-related oxidative stress in modulating EPC number and function has previously been implied, although the molecular effects of disease on EPC gene expression remain unclear. Sorrentino *et al*. (111) show increased levels of superoxide in EPC of type 2 diabetes mellitus (DM) patients, and concomitantly reduced availability of nitric oxide (NO) in EPCs. The EPCs from patients show a dramatically reduced re-endothelialization capacity in a carotid-injury model.

Patients with severe arterial disease are the primary target population for regenerative cell therapy, which currently most often involves autologous BM-derived cell administration (8, 29, 81, 112). However, because of the impaired regenerative potential of EPCs in these patients, therapeutic effectiveness of such cell therapy is likely to be suboptimal. Importantly, previous studies have demonstrated that EPC dysfunction is at least in part reversible. A number of compounds have been shown to improve the EPC functional capacity or to promote their mobilization, such as antioxidants (52), statins (138), erythropoietin (EPO) (44, 136), and PPAR-γ agonists (111, 134). The mechanisms by which these compounds act on EPCs are incompletely understood, as some act through pleiotropic effects rather than the intended targets for which the drugs have been designed. Transcriptional profiling could elucidate effects of the previously mentioned compounds on the entire EPC transcriptome. Moreover, transcriptome analysis could identify EPC gene clusters with differential expression between patients and healthy controls and facilitate the selection of novel, more-specific therapeutic targets.

The vast majority of studies on EPC transcriptomes in healthy and diseased conditions are conducted in CACs. Fiorito *et al*. (34) used microarrays to study the effects of tumor necrosis factor (TNF)-α and antioxidants vitamin C and E on CAC gene expression. The authors identified a small set of genes modulated by TNF-α or the antioxidants, although they stated that the biologic significance of these genes in the growth, survival, differentiation, and function of EPCs should be determined by further investigation.

Furthermore, two studies investigated the gene-expression profiles of CACs in type 1 DM patients compared with healthy controls (74, 126). Within the modulated gene set, both studies reported increased expression of antioxidant genes in diabetic versus healthy CACs, presumably as a result of diabetes-induced increase in oxidative stress levels. Interestingly, the latter study further demonstrated that folic acid supplementation altered gene-expression profiles of CACs in patients to resemble those of healthy subjects. The effects of EPO on monocyte and CAC gene-expression profiles from patients with combined renal and heart failure are currently being evaluated in the EPOCARES clinical trial (125).

To our knowledge, Tan *et al*. (117) are the first to compare ECFCs from patients with proliferative diabetic retinopathy (PDR) and healthy controls at the transcriptome level. The authors found a number of genes to be upregulated in ECFCs of PDR patients, including two known angiogenesis inhibitors, thrombospondin-1 and tissue inhibitor of matrix me-

talloproteinases-3 (TIMP-3), possibly explaining (part of) the vascular complications in diabetes patients.

Transcriptome analysis of BM samples obtained from patients receiving autologous cell therapy may provide information to predict the response to therapy. Comparing responders and non-responders to autologous EPC therapy could identify signature genes that correlate with functional improvement, while also providing insight into molecular mechanisms of EPC-mediated neovascularization. In an analogous example, Schirmer *et al*. (106) compared transcriptomes of circulating naïve (monocytes, T-cells, $CD34^+$ cells) and stimulated (LPS-stimulated monocytes and plastic-adherent monocytes/macrophages) cells of arteriogenic responders and non-responders in a patient population with coronary artery disease. Overall, the authors found that in nonresponders, arteriogenesis is inhibited rather than that it is enhanced in responders, as they had hypothesized. Most pronounced differences were found in the stimulated cells, with monocytes from nonresponders expressing more interferon beta after stimulation with LPS. Subsequent experiments showed that interferon beta inhibited the arteriogenic response in a murine hindlimb-ischemia model (106). Taken together, these studies illustrate that transcriptome profiling can help to unveil key mechanisms that are modulated by disease in EPCs. Knowledge thus obtained may contribute to ameliorating EPC dysfunction and optimizing EPC therapy.

Conclusion and Perspectives

At present, transcriptome profiling is a widely used tool for the analysis of gene-expression differences between different cells, treatments, or populations. In this review, we summarized EPC transcriptome analyses to serve as a base for understanding the identity, differentiation, and pathophysiology of different EPC types currently described in the literature. Our meta-analysis of transcriptome studies of cells from the hematopoietic and endothelial lineages demonstrates that transcriptomes can be used for classification and characterization of a particular cell in an unbiased manner. This approach is therefore a powerful addition to characterization based on cell-surface markers only. Once a cell has been characterized, signature genes can be identified from comparative analysis, allowing subsequent easy and high-throughput analyses (77). The currently available transcriptome datasets are few in number, making meta-analyses underpowered, and are hampered by the fact that they are performed by different groups, by using cells from different donors analyzed on different platforms. A direct comparison of transcriptomes from different EPC types to related circulating cells would be a more-promising approach to enable the identification of cell-specific properties and marker genes, yielding valuable information about EPC biology.

The acquired information may prove to be crucial for future therapeutic use of EPCs. Insight into the ontogeny will provide tools for the optimization of cell selection, culturing, and treatment for therapeutic use. Based on the identification of therapeutically relevant EPC types, endogenous cells may be selectively isolated or targeted. For example, the low efficiency and relatively long expansion period to obtain ECFCs may be improved by selective isolation based on specific cell-surface markers identified in gene-expression profiling studies (64).

Although gene-expression profiles provide insight into the (patho)physiological condition of a cell or tissue at a given moment, many biologic effects do not take place at the transcriptional level. For example, protein abundance does not necessarily correlate with mRNA levels. The secretion of growth factors and other substances is regulated mainly at the protein level, depending on intracellular trafficking and phosphorylation (22, 28). Currently, analyses of biological processes by using proteomics and metabolomics approaches are becoming increasingly widespread (10, 80, 113), and analyses of gene-expression regulation by microRNAs increasingly enter the public domain (84, 147). Fleissner *et al*. (34) recently used an array-based approach to identify microRNAs expressed in CACs and were able to illustrate the role of miR-21 in asymmetric dimethyl arginine (ADMA)-mediated CAC dysfunction. It was shown that miR-21 downregulates the expression of MnSOD, and that levels of miR-21 negatively correlate with migratory capacity in CACs isolated from patients with CAD (34).

Medina *et al*. (82) included a proteomic approach in their ECFC versus CAC comparison, and the proteomics analysis by Prokopi *et al.* (94) revealed a previously unknown mechanism for the acquisition of endothelial antigens by CACs. An article by the Mayr group (95) describes the identification of secreted thymidine phosphorylase as an important paracrine factor in the stimulation of angiogenesis by CACs. These studies illustrate the power of proteomics as an additional tool for cell characterization, and hint about integrating analyses of secreted products by proteomic and metabolomic approaches, based on the transcriptome analyses discussed in this review.

Altogether, current and future efforts toward the comprehensive profiling of EPC mRNA, protein, and metabolites will provide detailed insight into the biology, function, and potential therapeutic applications of EPCs.

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References

1. This reference has been deleted.
2. Alizadeh AA, Eisen MB, Davis RE, Ma C, and Lossos IS. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 403: 503–511, 2000.
3. Arai M, Misao Y, Nagai H, Kawasaki M, and Nagashima K. Granulocyte colony-stimulating factor: a noninvasive regeneration therapy for treating atherosclerotic peripheral artery disease. *Circ J* 70: 1093–1098, 2006.
4. Asahara T, Masuda H, Takahashi T, Kalka C, and Pastore C. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 85: 221–228, 1999.

5. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, *et al*. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275: 964–966, 1997.
6. Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, *et al*. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. *EMBO J* 18: 3964–3972, 1999.
7. Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, *et al*. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet* 362: 697–703, 2003.
8. Assmus B, Honold J, Schachinger V, Britten MB, Fischer-Rasokat U, *et al*. Transcoronary transplantation of progenitor cells after myocardial infarction. *N Engl J Med* 355: 1222–1232, 2006.
9. Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, *et al*. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI) *Circulation* 106: 3009–3917, 2002.
10. Becker RC and Andreotti F. Preoteomics, metabolomics and progenitor cells in acute coronary syndromes. *J Thromb Thrombol* 22: 85–88, 2006.
11. Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, *et al*. Minimum information about a microarray experiment (MIAME) toward standards for microarray data. *Nat Genet* 29: 365–371, 2001.
12. Breitling R, Armengaud P, Amtmann A, and Herzyk P. Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. *FEBS Lett* 573: 83–92, 2004.
13. Bryder D, Rossi DJ and Weissman IL. Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. *Am J Pathol* 169: 338–346, 2006.
14. Capoccia BJ, Robson DL, Levac KD, Maxwell DJ, Hohm SA, *et al*. Revascularization of ischemic limbs after transplantation of human bone marrow cells with high aldehyde dehydrogenase activity. *Blood* 113: 5340–5351, 2009.
15. Carmeliet P. Angiogenesis in life, disease and medicine. *Nature* 438: 932–936, 2005.
16. Casamassimi A, Balestrieri ML, Fiorito C, Schiano C, Maione C, *et al*. Comparison between total endothelial progenitor cell isolation versus enriched Cd133+ culture. *J Biochem* 141: 503–511, 2007.
17. Case J, Mead LE, Bessler WK, Prater D, White HA, *et al*. Human CD34+ AC133+ VEGFR-2+ cells are not endothelial progenitor cells but distinct, primitive hematopoietic progenitors. *Exp Hematol* 35: 1109–1118, 2007.
18. Chavakis E, Aicher A, Heeschen C, Sasaki Ki, Kaiser R, *et al*. Role of beta2-integrins for homing and neovascularization capacity of endothelial progenitor cells. *J Exp Med* 201: 63–72, 2005.
19. Chavakis E, Koyanagi M, and Dimmeler S. Enhancing the outcome of cell therapy for cardiac repair: progress from bench to bedside and back. *Circulation* 121: 325–335, 2010.
20. Chen H, Campbell RA, Chang Y, Li M, Wang CS, *et al*. Pleiotrophin produced by multiple myeloma induces transdifferentiation of monocytes into vascular endothelial cells: a novel mechanism of tumor-induced vasculogenesis. *Blood* 113: 1992–2002, 2009.
21. Chen JZ, Zhang FR, Tao QM, Wang XX, Zhu JH, and Zhu JH. Number and activity of endothelial progenitor cells from peripheral blood in patients with hypercholesterolaemia. *Clin Sci* 107: 273–280, 2004.
22. Chen LA, Li J, Silva SR, Jackson LN, Zhou Y, *et al*. PKD3 is the predominant protein kinase D isoform in mouse exocrine pancreas and promotes hormone-induced amylase secretion. *J Biol Chem* 284: 2459–2471, 2009.
23. Deb A and Patterson C. Hard luck stories: the reality of endothelial progenitor cells continues to fall short of the promise. *Circulation* 121: 850–852, 2010.
24. Dennis G, Sherman B, Hosack D, Yang J, Gao W, *et al*. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* 4: 3, 2003.
25. Dernbach E, Urbich C, Brandes RP, Hofmann WK, Zeiher AM, and Dimmeler S. Antioxidative stress-associated genes in circulating progenitor cells: evidence for enhanced resistance against oxidative stress. *Blood* 104: 3591–3597, 2004.
26. Desai A, Glaser A, Liu D, Raghavachari N, Blum A, *et al*. Microarray-based characterization of a colony assay used to investigate endothelial progenitor cells and relevance to endothelial function in humans. *Arterioscler Thromb Vasc Biol* 29: 121–127, 2009.

26a. Editors comment: To affinity . . . and beyond! *Nat Genet* 14: 367–370, 1996.

27. Elsheikh E, Uzunel M, He Z, Holgersson J, Nowak G, and Sumitran-Holgersson S. Only a specific subset of human peripheral-blood monocytes has endothelial-like functional capacity. *Blood* 106: 2347–2355, 2005.
28. Esper RM and Loeb JA. Neurotrophins induce neuregulin release through protein kinase Cdelta activation. *J Biol Chem* 284: 26251–26260, 2009.
29. Fadini GP, Agostini C, and Avogaro A. Autologous stem cell therapy for peripheral arterial disease: meta-analysis and systematic review of the literature. *Atherosclerosis* 209: 10–17, 2010.
30. Fadini GP, Coracina A, Baesso I, Agostini C, Tiengo A, *et al*. Peripheral blood CD34+ KDR+ endothelial progenitor cells are determinants of subclinical atherosclerosis in a middle-aged general population. *Stroke* 37: 2277–2282, 2006.
31. Fadini GP, Miorin M, Facco M, Bonamico S, Baesso I, *et al*. Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus. *J Am Coll Cardiol* 45: 1449–1457, 2005.
32. Fadini GP, Sartore S, Albiero M, Baesso I, Murphy E, *et al*. Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy. *Arterioscler Thromb Vasc Biol* 26: 2140–2146, 2006.
33. Fernandez Pujol B, Lucibello FC, Gehling UM, Lindemann K, Weidner N, *et al*. Endothelial-like cells derived from human CD14 positive monocytes. *Differentiation* 65: 287–300, 2000.
34. Fiorito C, Rienzo M, Crimi E, Rossiello R, Luisa Balestrieri M, *et al*. Antioxidants increase number of progenitor endothelial cells through multiple gene expression pathways. *Free Radic Res* 42: 754–762, 2008.
35. Florey HW, Greer SJ, Poole JC, and Werthessen NT. The pseudointima lining fabric grafts of the aorta. *Br J Exp Pathol* 42: 236–246, 1961.
36. Fodor SPA, Read JL, Pirrung MC, Stryer L, Lu AT, and Solas D. Light-directed, spatially addressable parallel chemical synthesis. *Science* 251: 767–773, 1991.
37. Folkman J. What is the role of endothelial cells in angiogenesis? *Lab Invest* 51: 601–604, 1984.
38. Fuchs S, Hermanns MI and Kirkpatrick CJ. Retention of a differentiated endothelial phenotype by outgrowth endothelial cells isolated from human peripheral blood and expanded in long-term cultures. *Cell Tissue Res* 326: 79–92, 2006.

39. Garlanda C and Dejana E. Heterogeneity of endothelial cells: specific markers. *Arterioscler Thromb Vasc Biol* 17: 1193–1202, 1997.
40. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, *et al*. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* 286: 531–537, 1999.
41. Grunewald M, Avraham I, Dor Y, Bachar-Lustig E, Itin A, *et al*. VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. *Cell* 124: 175–189, 2006.
42. Hagensen MK, Shim J, Thim T, Falk E, and Bentzon JF. Circulating endothelial progenitor cells do not contribute to plaque endothelium in murine atherosclerosis. *Circulation* 121: 898–905, 2010.
43. Hankey GJ, Norman PE, and Eikelboom JW. Medical treatment of peripheral arterial disease. *JAMA* 295: 547–553, 2006.
44. Heeschen C, Aicher A, Lehmann R, Fichtlscherer S, Vasa M, *et al*. Erythropoietin is a potent physiologic stimulus for endothelial progenitor cell mobilization. *Blood* 102: 1340–1346, 2003.
45. Heeschen C, Lehmann R, Honold J, Assmus B, Aicher A, *et al*. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. *Circulation* 109: 1615–1622, 2004.
46. Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, *et al*. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell* 109: 625–637, 2002.
47. Higashi Y, Kimura M, Hara K, Noma K, Jitsuiki D, *et al*. Autologous bone-marrow mononuclear cell implantation improves endothelium-dependent vasodilation in patients with limb ischemia. *Circulation* 109: 1215–1218, 2004.
48. Hill JM, Zalos G, Halcox JPJ, Schenke WH, Waclawiw MA, *et al*. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 348: 593–600, 2003.
49. Hirsch A, Nijveldt R, van der Vleuten PA, Biemond BJ, Doevendans PA, *et al*. Intracoronary infusion of autologous mononuclear bone marrow cells or peripheral mononuclear blood cells after primary percutaneous coronary intervention: rationale and design of the HEBE trial: a prospective, multicenter, randomized trial. *Am Heart J* 152: 434–441, 2006.
50. Hong F, Breitling R, McEntee CW, Wittner BS, Nemhauser JL, and Chory J. RankProd: a bioconductor package for detecting differentially expressed genes in meta-analysis. *Bioinformatics* 22: 2825–2827, 2006.
51. Huang P, Li S, Han M, Xiao Z, Yang R, and Han ZC. Autologoust transplantation of granulocyte colony stimulating factor μobilized peripheral blood mononuclear cells improves critical limb ischemia in diabetes. *Diabetes Care* 28: 2155–2160, 2005.
52. Huang PH, Chen YH, Tsai HY, Chen JS, Wu TC, *et al*. Intake of red wine increases the number and functional capacity of circulating endothelial progenitor cells by enhancing nitric oxide bioavailability. *Arterioscler Thromb Vasc Biol* 30: 869–877, 2010.
53. Huang PH, Chen YH, Wang CH, Chen JS, Tsai HY, *et al*. Matrix metalloproteinase-9 is essential for ischemia-induced neovascularization by modulating bone marrow-derived endothelial progenitor cells. *Arterioscler Thromb Vasc Biol* 29: 1179–1184, 2009.
54. Hume DA, Summers KM, Raza S, Baillie JK, and Freeman TC. Functional clustering and lineage markers: insights into cellular differentiation and gene function from large-scale microarray studies of purified primary cell populations. *Genomics* 2010 (in press).
55. Hur J, Yoon CH, Kim HS, Choi JH, Kang HJ, *et al*. Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. *Arterioscler Thromb Vasc Biol* 24: 288–293, 2004.
56. Igreja C, Fragoso R, Caiado F, Clode N, Henriques A, *et al*. Detailed molecular characterization of cord blood-derived endothelial progenitors. *Exp Hematol* 36: 193, 2008
57. Ingram DA, Mead LE, Tanaka H, Meade V, Fenoglio A, *et al*. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood* 104: 2752–2760, 2004.
58. Ishida A, Ohya Y, Sakuda H, Ohshiro K, Higashiuesato Y, *et al*. Autologous peripheral blood mononuclear cell implantation for patients with peripheral arterial disease improves limb ischemia. *Circ J* 69: 1260–1265, 2005.
59. Jain AK, Murty MN, and Flynn PJ. Data clustering: a review. *ACM Comput Surv* 31: 264–323, 1999.
60. Jie KE, Zaikova MA, Bergevoet MWT, Westerweel PE, Rastmanesh M, *et al*. Progenitor cells and vascular function are impaired in patients with chronic kidney disease. *Nephrol Dial Transplant* 25: 1875–1882, 2010.
61. Kalka C, Masuda H, Takahashi T, Kalka-Moll WM, Silver M, *et al*. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. *Proc Natl Acad Sci U S A* 97: 3422–3427, 2000
62. Kennedy LJ Jr and Weissman IL. Dual origin of intimal cells in cardiac-allograft arteriosclerosis. *N Engl J Med* 285: 884–887, 1971.
63. Kerr G, Ruskin HJ, Crane M, and Doolan P. Techniques for clustering gene expression data. *Comput Biol Med* 38: 283–293, 2008.
64. Kolbe M, Dohle E, Katerla D, Kirkpatrick CJ, and Fuchs S. Enrichment of outgrowth endothelial cells in high and low colony-forming cultures from peripheral blood progenitors. *Tissue Eng Part C Methods* 16: 877–886, 2010.
65. Krenning G, van der Strate BW, Schipper M, van Seijen XJ, Fernandes BC, *et al*. CD34+ cells augment endothelial cell differentiation of CD14+ endothelial progenitor cells in vitro. *J Cell Mol Med* 13: 2521–2533, 2009.
66. Kuki S, Imanishi T, Kobayashi K, Matsuo Y, Obana M, and Akasaka T. Hyperglycemia accelerated endothelial progenitor cell senescence via the activation of p38 mitogen-activated protein kinase. *Circ J* 70: 1076–1081, 2006.
67. Lancrin C, Sroczynska P, Serrano A, Gandillet A, Ferreras C, *et al*. Blood cell generation from the hemangioblast. *J Mol Med* 88: 167–172, 2010.
68. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, *et al*. Initial sequencing and analysis of the human genome. *Nature* 409: 860–921, 2001.
69. Li B, Cohen A, Hudson TE, Motlagh D, Amrani DL, and Duffield JS. Mobilized human hematopoietic stem/progenitor cells promote kidney repair after ischemia/reperfusion injury. *Circulation* 121: 2211–2220, 2010.
70. Libby P. Inflammation in atherosclerosis. *Nature* 420: 868–874, 2002.
71. Lin Y, Weisdorf DJ, Solovey A, and Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest* 105: 71–77, 2000.
72. Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, *et al*. Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat Biotech* 14: 1675–1680, 1996.

73. Lockhart DJ and Winzeler EA. Genomics, gene expression and DNA arrays. *Nature* 405: 827–836, 2000.
74. Loomans CJM, De Koning EJP, Staal FJT, Rabelink TJ, and Zonneveld AJV. Endothelial progenitor cell dysfunction in type 1 diabetes: another consequence of oxidative stress? *Antioxid Redox Signal* 7: 1468–1475, 2005.
75. Loomans CJM, de Koning EJP, Staal FJT, Rookmaaker MB, Verseyden C, *et al*. Endothelial progenitor cell dysfunction. *Diabetes* 53: 195–199, 2004.
76. Lukk M, Kapushesky M, Nikkila J, Parkinson H, Goncalves A, *et al*. A global map of human gene expression. *Nat Biotech* 28: 322–324, 2010.
77. MacArthur BD, Tare RS, Murawski K, and Oreffo RO. Identification of candidate regulators of multipotency in human skeletal progenitor cells. *Biochem Biophys Res Commun* 377: 68–72, 2008.
78. Mackay J and Mensah G. *The Atlas of Heart Disease and Stroke.* Geneva: World Health Organization, 2004.
79. Mackenzie DC and Loewenthal J. Endothelial growth in nylon vascular grafts. *Br J Surg* 48: 212–217, 1960.
80. Maletic-Savatic M, Vingara LK, Manganas LN, Li Y, Zhang S, *et al*. Metabolomics of neural progenitor cells: a novel approach to biomarker discovery. *Cold Spring Harb Symp Quant Biol* 73: 389–401, 2008.
81. Martin-Rendon E, Brunskill S, Doree C, Hyde C, Watt S, *et al*. Stem cell treatment for acute myocardial infarction. *Cochrane Database Syst Rev* CD006536, 2008.
82. Medina R, O'Neill C, Sweeney M, Guduric-Fuchs J, Gardiner T, *et al*. Molecular analysis of endothelial progenitor cell (EPC) subtypes reveals two distinct cell populations with different identities. *BMC Med Genomics* 3: 18, 2010.
83. Michowitz Y, Goldstein E, Wexler D, Sheps D, Keren G, and George J. Circulating endothelial progenitor cells and clinical outcome in patients with congestive heart failure. *Heart* 93: 1046–1050, 2007.
84. Minami Y, Satoh M, Maesawa C, Takahashi Y, Tabuchi T, *et al*. Effect of atorvastatin on microRNA 221/222 expression in endothelial progenitor cells obtained from patients with coronary artery disease. *Eur J Clin Invest* 39: 359–367, 2009.
85. Mukai N, Akahori T, Komaki M, Li Q, Kanayasu-Toyoda T, *et al*. A comparison of the tube-forming potentials of early and late endothelial progenitor cells. *Exp Cell Res* 314: 430–440, 2008.
86. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, and Fowkes FGR. Inter-society consensus for the management of peripheral arterial disease (TASC II). *J Vasc Surg* 45: S5–S67, 2007.
87. Otten J, Schultze A, Schafhausen P, Otterstetter S, Dierlamm J, *et al*. Blood outgrowth endothelial cells from chronic myeloid leukaemia patients are BCR/ABL1 negative. *Br J Haematol* 142: 115–118, 2008.
88. Peeters SDPW, van der Kolk DM, de Haan G, Bystrykh L, Kuipers F, *et al*. Selective expression of cholesterol metabolism genes in normal CD34+ CD38- cells with a heterogeneous expression pattern in AML cells. *Exp Hematol* 34: 622–630, 2006.
89. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, *et al*. Expression of VEGFR-2 and AC133 by circulating human CD34+ cells identifies a population of functional endothelial precursors. *Blood* 95: 952–958, 2000.
90. Peled A, Kollet O, Ponomaryov T, Petit I, Franitza S, *et al*. The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34+ cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. *Blood* 95: 3289–3296, 2000
91. Piaggio G, Rosti V, Corselli M, Bertolotti F, Bergamaschi G, *et al*. Endothelial colony-forming cells from patients with chronic myeloproliferative disorders lack the disease-specific molecular clonality marker. *Blood* 114: 3127–3130, 2009.
92. Poole JC, Sabiston DC Jr, Florey HW, and Allison PR. Growth of endothelium in arterial prosthetic grafts and following endarterectomy. *Surg Forum* 13: 225–227, 1962.
93. Prater DN, Case J, Ingram DA, and Yoder MC. Working hypothesis to redefine endothelial progenitor cells. *Leukemia* 21: 1141–1149, 2007.
94. Prokopi M, Pula G, Mayr U, Devue C, Gallagher J, *et al*. Proteomic analysis reveals presence of platelet microparticles in endothelial progenitor cell cultures. *Blood* 114: 723–732, 2009.
95. Pula G, Mayr U, Evans C, Prokopi M, Vara DS, *et al*. Proteomics identifies thymidine phosphorylase as a key regulator of the angiogenic potential of colony-forming units and endothelial progenitor cell cultures. *Circ Res* 104: 32–40, 2009.
96. Rehman J, Li J, Orschell CM, and March KL. Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation* 107: 1164–1169, 2003.
97. Reinisch A, Hofmann NA, Obenauf AC, Kashofer K, Rohde E, *et al*. Humanized large-scale expanded endothelial colony-forming cells function in vitro and in vivo. *Blood* 113: 6716–6725, 2009.
98. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, *et al*. Large-scale meta-analysis of cancer microarray data identifies common transcriptional profiles of neoplastic transformation and progression. *Proc Natl Acad Sci U S A* 101: 9309–9314, 2004.
99. Risau W. Mechanisms of angiogenesis. *Nature* 386: 671–674, 1997.
100. Risau W and Flamme I. Vasculogenesis. *Annu Rev Cell Dev Biol* 11: 73–91, 1995.
101. Rohde E, Bartmann C, Schallmoser K, Reinisch A, Lanzer G, *et al*. Immune cells mimic the morphology of endothelial progenitor colonies in vitro. *Stem Cells* 25: 1746–1752, 2007.
102. Romagnani P, Annunziato F, Liotta F, Lazzeri E, Mazzinghi B, *et al*. CD14+ CD34 low cells with stem cell phenotypic and functional features are the major source of circulating endothelial progenitors. *Circ Res* 97: 314–322, 2005.
103. Rookmaaker MB, Vergeer M, van Zonneveld AJ, Rabelink TJ, Verhaar MC, *et al*. Endothelial progenitor cells: mainly derived from the monocyte/macrophage-containing CD34- mononuclear cell population and only in part from the hematopoietic stem cell-containing CD34+ mononuclear cell population response. *Circulation* 108: e150, 2003.
104. Ruegg C, Tissot JD, Farmer P, and Mariotti A. Omics meets hypothesis-driven research: partnership for innovative discoveries in vascular biology and angiogenesis. *Thromb Haemost* 100: 738–746, 2008.
105. Sara H, Kallioniemi O, and Nees M. A decade of cancer gene profiling: from molecular portraits to molecular function. *Methods Mol Biol* 576: 61–87, 2010.
106. Schirmer SH, Fledderus JO, Bot PTG, Moerland PD, Hoefer IE, *et al*. Interferon-{beta} signaling Is enhanced in patients with insufficient coronary collateral artery development and inhibits arteriogenesis in mice. *Circ Res* 102: 1286–1294, 2008.
107. Schmeisser A, Garlichs CD, Zhang H, Eskafi S, Graffy C, *et al*. Monocytes coexpress endothelial and macrophagocytic

lineage markers and form cord-like structures in Matrigel under angiogenic conditions. *Cardiovasc Res* 49: 671–680, 2001.
108. Schmidt-Lucke C, Rossig L, Fichtlscherer S, Vasa M, Britten M, *et al*. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair. *Circulation* 111: 2981–2987, 2005.
109. Seeger FH, Tonn T, Krzossok N, Zeiher AM, and Dimmeler S. Cell isolation procedures matter: a comparison of different isolation protocols of bone marrow mononuclear cells used for cell therapy in patients with acute myocardial infarction. *Eur Heart J* 28: 766–772, 2007.
110. Sieveking DP, Buckle A, Celermajer DS, and Ng MKC. Strikingly different angiogenic properties of endothelial progenitor cell subpopulations: insights from a novel human angiogenesis assay. *J Am Coll Cardiol* 51: 660–668, 2008.
111. Sorrentino SA, Bahlmann FH, Besler C, Muller M, Schulz S, *et al*. Oxidant stress impairs in vivo reendothelialization capacity of endothelial progenitor cells from patients with type 2 diabetes mellitus: restoration by the peroxisome proliferator-activated receptor-{gamma} agonist rosiglitazone. *Circulation* 116: 163–173, 2007.
112. Sprengers RW, Moll FL, Teraa M, and Verhaar MC. Rationale and design of the JUVENTAS trial for repeated intra-arterial infusion of autologous bone marrow-derived mononuclear cells in patients with critical limb ischemia. *J Vasc Surg* 51: 1564–1568, 2010.
113. Stastna M, Abraham MR, and Van Eyk JE. Cardiac stem/progenitor cells, secreted proteins, and proteomics. *FEBS Lett* 583: 1800–1807, 2009.
114. Strauer BE, Brehm M, Zeus T, Kostering M, Hernandez A, *et al*. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 106: 1913–1918, 2002.
115. Stump MM, Jordan GL Jr, Debakey ME, and Halpert B. Endothelium grown from circulating blood on isolated intravascular dacron hub. *Am J Pathol* 43: 361–367, 1963.
116. Takahashi T, Kalka C, Masuda H, Chen D, Silver M, *et al*. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med* 5: 434–438, 1999.
117. Tan K, Lessieur E, Cutler A, Nerone P, Vasanji A, *et al*. Impaired function of circulating CD34+ CD45- cells in patients with proliferative diabetic retinopathy. *Exp Eye Res* 2010 (in press).
118. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, *et al*. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet* 360: 427–435, 2002.
119. Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, *et al*. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 106: 2781–276, 2002.
120. Timmermans F, Plum J, Yoder MC, Ingram DA, Vandekerckhove B, and Case J. Endothelial progenitor cells: identity defined? *J Cell Mol Med* 13: 87–102, 2009.
121. Urbich C, Aicher A, Heeschen C, Dernbach E, Hofmann WK, *et al*. Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. *J Mol Cell Cardiol* 39: 733–742, 2005.
122. Urbich C, Heeschen C, Aicher A, Dernbach E, Zeiher AM, and Dimmeler S. Relevance of monocytic features for neovascularization capacity of circulating endothelial progenitor cells. *Circulation* 108: 2511–2516, 2003.
123. Urbich C, Heeschen C, Aicher A, Sasaki Ki, Bruhl T, *et al*. Cathepsin L is required for endothelial progenitor cell-induced neovascularization. *Nat Med* 11: 206–213, 2005.
124. van Beem RT, Noort WA, Voermans C, Kleijer M, ten Brinke A, *et al*. The presence of activated CD4+ T cells is essential for the formation of colony-forming unit-endothelial cells by CD14+ cells. *J Immunol* 180: 5141–5148, 2008.
125. van der Putten K, Jie KE, Emans ME, Verhaar MC, Joles JA, *et al*. Erythropoietin treatment in patients with combined heart and renal failure: objectives and design of the EPO-CARES study. *J Nephrol* 2010 (in press).
126. van Oostrom O, de Kleijn D, Fledderus J, Pescatori M, Stubbs A, *et al*. Folic acid supplementation normalizes the endothelial progenitor cell transcriptome of patients with type 1 diabetes: a case-control pilot study. *Cardiovasc Diabetol* 8: 47, 2009.
127. van Royen N, Schirmer SH, Atasever B, Behrens CYH, Ubbink D, *et al*. START Trial: a pilot study on sTimulation of ARTeriogenesis using subcutaneous application of granulocyte-macrophage colony-stimulating factor as a new treatment for peripheral vascular disease. *Circulation* 112: 1040–1046, 2005.
128. Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, *et al*. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 89: E1–E7, 2001.
129. Vasa M, Fichtlscherer S, Adler K, Aicher A, Martin H, *et al*. Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. *Circulation* 103: 2885–2890, 2001.
130. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, *et al*. The sequence of the human genome. *Science* 291: 1304–1351, 2001.
131. Walter DH, Haendeler J, Reinhold J, Rochwalsky U, Seeger F, *et al*. Impaired CXCR4 signaling contributes to the reduced neovascularization capacity of endothelial progenitor cells from patients with coronary artery disease. *Circ Res* 97: 1142–1151, 2005.
132. Watkins NA, Gusnanto A, de Bono B, De S, Miranda-Saavedra D, *et al*. A haematlas: characterizing gene expression in differentiated human blood cells. *Blood* 113: e1–e9, 2009.
133. Weigelt B and Reis-Filho JS. Histological and molecular types of breast cancer: is there a unifying taxonomy? *Nat Rev Clin Oncol* 6: 718–730, 2009.
134. Werner C, Kamani CH, Gensch C, Bahm M, and Laufs U. The peroxisome proliferator activated receptor-agonist pioglitazone increases number and function of endothelial progenitor cells in patients with coronary artery disease and normal glucose tolerance. *Diabetes* 56: 2609–2615, 2007.
135. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, *et al*. Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med* 353: 999–1007, 2005.
136. Westenbrink BD, Lipic E, van der Meer P, van der Harst P, Oeseburg H, *et al*. Erythropoietin improves cardiac function through endothelial progenitor cell and vascular endothelial growth factor mediated neovascularization. *Eur Heart J* 28: 2018–2027, 2007.
137. Westerweel PE, den OK, Nguyen TQ, Goldschmeding R, Joles JA, and Verhaar MC. Amelioration of anti-Thy1-glomerulonephritis by PPAR-gamma agonism without increase of endothelial progenitor cell homing. *Am J Physiol Renal Physiol* 294: F379–F384, 2008.

138. Westerweel PE, Visseren FL, Hajer GR, Olijhoek JK, Hoefer IE, *et al*. Endothelial progenitor cell levels in obese men with the metabolic syndrome and the effect of simvastatin monotherapy vs. simvastatin/ezetimibe combination therapy. *Eur Heart J* 29: 2808–2817, 2008.
139. Winnepenninckx V, Lazar V, Michiels S, Dessen P, Stas M, *et al*. Gene expression profiling of primary cutaneous melanoma and clinical outcome. *J Natl Cancer Inst* 98: 472–482, 2006.
140. Writing Group, Rosamond W, Flegal K, Furie K, Go A, *et al*. Heart disease and stroke statistics-2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 117: e25–e146, 2008.
141. Yoder MC. Defining human endothelial progenitor cells. *J Thromb Haemost* 7 Suppl 1: 49–52, 2009.
142. Yoder MC, Mead LE, Prater D, Krier TR, Mroueh KN, *et al*. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* 109: 1801–1809, 2007.
143. Yoon CH, Hur J, Park KW, Kim JH, Lee CS, *et al*. Synergistic neovascularization by mixed transplantation of early endothelial progenitor cells and late outgrowth endothelial cells: the role of angiogenic cytokines and matrix metalloproteinases. *Circulation* 112: 1618–1627, 2005.
144. Zeisberger SM, Zoller S, Riegel M, Chen S, Krenning G, *et al*. Optimization of the culturing conditions of human umbilical cord blood-derived endothelial colony-forming cells under xeno-free conditions applying a transcriptomic approach. *Genes Cells* 15: 671–687, 2010.
145. Zemani F, Silvestre JS, Fauvel-Lafeve F, Bruel A, Vilar J, *et al*. Ex vivo priming of endothelial progenitor cells with SDF-1 before transplantation could increase their proangiogenic potential. *Arterioscler Thromb Vasc Biol* 28: 644–650, 2008.
146. Zhang SJ, Zhang H, Wei YJ, Su WJ, Liao ZK, *et al*. Adult endothelial progenitor cells from human peripheral blood maintain monocyte/macrophage function throughout in vitro culture. *Cell Res* 16: 577–584, 2006.
147. Zhao T, Li J, and Chen AF. MicroRNA-34a induces endothelial progenitor cell senescence and impedes its angiogenesis via suppressing silent information regulator 1. *Am J Physiol Endocrinol Metab* 299: E110–E116, 2010.

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Abbreviations Used

ADMA = asymmetric dimethylarginine
BM = bone marrow
CAC = circulating angiogenic cell
Cat = catalase
CD = cluster of differentiation
cEPC = circulating endothelial progenitor cell
CFU = colony-forming unit
CML = chronic myeloid leukemia
CXCR4 = CXC chemokine receptor 4
Dll4 = delta-like ligand 4
DM = diabetes mellitus
ECFE = endothelial colony-forming cell
ECM = extracellular matrix
eNOS = endothelial nitric oxide synthase
EPC = endothelial progenitor cell
EPO = erythropoietin
GPX = glutathione peroxidase
HGF = hepatocyte growth factor
HIF = hypoxia-inducible factor
HLA = human leukocyte antigen (major histocompatibility complex)
HMEC = human microvascular endothelial cell
HOX = homeobox
HUVEC = human umbilical vein endothelial cell
IGF = insulin-like growth factor
IL = interleukin
KDR = kinase insert domain receptor
LDL = low-density lipoprotein
LPS = lipopolysaccharide
LYN v-yes-1 = Yamaguchi sarcoma viral-related oncogene homologue
MMP = matrix metalloproteinase
MnSOD = manganese superoxide dismutase
NO = nitric oxide
PBMC = peripheral blood mononuclear cell
PCR = polymerase chain reaction
PDGF = platelet-derived growth factor
PDR = proliferative diabetic retinopathy
ROS = reactive oxygen species
RUNX1 = runt-related transcription factor 1
SDF = stromal cell–derived factor
TIMP-3 = tissue inhibitor of matrix metalloproteinases-3
TLR = Toll-like receptor
TNF = tumor necrosis factor
VEGF = vascular endothelium growth factor
VEGFR = vascular endothelium growth factor receptor
vWF = von Willebrand factor
WAS = Wiskott-Aldrich syndrome

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2. T.C.T.M. van der Pouw Kraan, A.M. van der Laan, J.J. Piek, A.J.G. Horrevoets. 2012. Surfing the data tsunami, a bioinformatic dissection of the proangiogenic monocyte. *Vascular Pharmacology* **56**:5-6, 297-305. [CrossRef]
3. Joyce Doorn , Guido Moll , Katarina Le Blanc , Clemens van Blitterswijk , Jan de Boer . 2012. Therapeutic Applications of Mesenchymal Stromal Cells: Paracrine Effects and Potential Improvements. *Tissue Engineering Part B: Reviews* **18**:2, 101-115. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
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5. Ralf P. Brandes , Masuko Ushio-Fukai . 2011. Endothelial Progenitor Cells=EPC=Elemental Pernicious Complexity. *Antioxidants & Redox Signaling* **15**:4, 911-914. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]