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Genetic Links Between Circulating Cells and Cardiovascular Risk

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While algorithms such as the Framingham Risk Score have been instrumental in helping to stratify cardiovascular disease (CVD) susceptibility, it is estimated that 15-20% of patients presenting with myocardial infarction (MI) may lack any history of traditional risk factors [1]. These and other observations have prompted investigators to develop additional biological and genetic assays that might improve risk prediction or capture risks that might be orthogonal to well known and traditional factors such as diabetes, smoking, hypercholesterolemia and hypertension. One promising but confusing area appears to be the interrogation of circulating cells that have been deemed endothelial progenitor cells (EPCs). In some studies, the levels of these circulating cells appear to provide predictive power about vascular function in healthy people [2, 3] and future cardiovascular events in patients with disease [4, 5]. It is tempting to speculate that the quantitative or functional assessment of cells in the circulation with angiogenic or vascular reparative properties might eventually provide a useful biological measurement that could aid in risk assessment. Yet, significant questions remain regarding the true nature of EPCs and the actual role of these cells in disease progression [6]. Answers to these and related questions are therefore urgently needed before the tantalizing promise of EPCs can be fully incorporated into any assessment of CVD risk.

The current manuscript by Shaw and colleagues in this issue of *Circulation-Cardiovascular Genetics* provides an elegant blueprint of how a combined biological and genetic approach might aid in our efforts to better understand EPC biology and ultimately better define and understand CVD risk [7]. The current study analyzed nearly 1800 participants in the Framingham Heart Study. For the most part, these individuals were healthy and free of overt cardiovascular disease. The authors performed a simple biological assay to determine the amount of early outgrowth colony forming units (CFUs) present in a fasting blood sample. The details of these CFUs will be discussed in more detail later, however previous work by this group and others have linked a decline in CFUs with increased risk of CVD [2, 8]. In the present study, the authors again found a significant, albeit relatively modest, association between CFUs and calculated Framingham Risk Score. In an effort to expand our understanding of this phenomenon, the authors next performed a genome wide association analysis to assess whether genetic polymorphisms might underlie the observed variations in

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CFU number. Two regions were subsequently identified as being associated with regulating CFU number in this population. These regions include the MOSC1 and the SLC22A3-LPAL2-LPA locus. Interestingly, the SLC22A3-LPAL2-LPA locus had been previously associated with MI risk [9]. Furthermore, in the current analysis the variant in this locus was associated with low CFU number ($P=4.9\times10^{-7}$) and increased MI risk ($P=1.1\times10^{-4}$). These observations therefore provide genetic support for the relationship made previously between CFU number and future cardiovascular events [4]. Furthermore, they suggest that a decline in the regenerative capacity or number of progenitor cells might provide a biological mechanism for the steep age-dependent rise in cardiovascular disease.

These current results build upon a large literature attempting to link subtypes of circulating cells to either subclinical disease or overt cardiovascular events. Such analysis has included genetic profiling of circulating monocytes in patients with and without disease [10]. Similarly, there is evidence that certain subtypes of monocytes ($CD14^{+}CD16^{+}$) might expand in patients with CVD [11, 12]. The majority of work regarding circulating cells and CVD risk has however centered on EPCs. Originally isolated by Asahara and colleagues, circulating EPCs were envisioned to be rare circulating cells of bone marrow origin that could from tube-like structures in culture and could be incorporated into vessels within areas of ischemia [13]. These cells appeared to be enriched in the $CD34^{+}$ and $Flk\text{-}1^{+}$ fraction of circulating mononuclear cells. After being plated on fibronectin-coated dishes, these presumably more primitive circulating cells appeared to manifest endothelial properties including the VEGF-stimulated release of nitric oxide, the uptake of both lectin Ulex Europeaus agglutinin-1 (UEA-1) and modified LDL, as well as the surface expression of a number of specific endothelial surface markers. This *in vitro* and *in vivo* phenotype led the authors to conclude these cells represented a circulating endothelial progenitor population that might underpin the capacity for vasculogenesis in the adult. Subsequently many laboratories have studied the biology and relevance of EPCs in a wide range of conditions. Two major approaches have been used to quantify the number of these cells [6]. The first takes advantage of cell surface markers and analyzes levels of presumptive EPC based on a combination of epitopes including CD34, AC133 and KDR (Flk-1 in rodents). One advantage of this technique is that it is quick and quantitative. On the other hand, the sole readout of a number can be misleading. As an example, it is important to note that for hematopoietic stem cells (HSCs), quantification of number and function may give discordant results. For instance, in many rodent models, the number of HSCs defined by cell surface epitopes, stays constant or actually increases with age. However, the activity of these HSCs (on a per cell basis) clearly declines as the animal ages [14].

The second approach to quantifying EPCs involves colony forming assays. Since the work of Asahara and colleagues, these colony forming assays have usually included a pre-plating step on fibronectin for either one [15] or two days [2], to remove monocytes and mature endothelial cells. Non-adherent cells remaining after the pre-plating step were subsequently cultured on fibronectin and colony number assessed seven to ten days later. These approaches were potentially more biological in that they required colony formation, a more functional output then abundance by flow cytometry. Nonetheless, they are labor intensive and prone to experimental variation based on a number of circumstances including culture conditions.

Using one or sometimes both of these approaches, there have been literally hundreds of studies correlating EPC number with specific clinical outcomes [6, 16]. Although initially conceived as potential cellular elements contributing to vasculogenesis, our observations that in the absence of overt disease, a decline in EPC numbers correlated with increased CVD risk, led us to speculate that EPCs may be more important in repair of existing vessels rather than in the formation of new blood vessels [2]. Unfortunately, while these studies are

intriguing, significant questions have arisen. Indeed, in the absence of a true definition of an EPC, different groups are likely measuring different cell populations. Similarly, even using colony based approaches, variations on how the assay is performed has led to order of magnitude variations between different groups with regard to reported abundance of EPCs in healthy people [2, 17]. This suggests that what one group calls or isolates as a colony is not necessarily the same as another group definition of the same presumed activity. More importantly, concerns have also been raised that using flow based cell surface identification or using colony based approaches, the cell being quantified is of hematopoietic not endothelial origin [6]. These concerns lead to a series of additional important issues. Included among these are: Do the two assay systems identify the same or different cell populations? Do the cells identified actually contribute to disease? If they do contribute, is this through the direct repair of existing blood vessels, through the ability to create new vessels, or do they alter disease progression by acting in an indirect paracrine fashion?

These are difficult questions to address using a strictly correlative approach of quantifying EPC number and assessing the presence or absence of a disease phenotype. In contrast, the manuscript by Shaw and colleagues provide a potential strategy to begin to get a handle on these perplexing issues. For instance, if the putative cell identified by flow cytometry or colony assay formation actually contributes to CVD risk, one would predict that genetic factors controlling EPC abundance could also be identified independently in non-biased GWAS for CVD. Reassuringly, as mentioned, the current study finds this to be the case. Indeed, since the SLC22A3-LPAL2-LPA locus previously identified to effect MI risk, also appears to regulate CFU number, these observations provide some assurance that the cells identified by colony formation are not biomarkers or epiphenomena but rather potentially intrinsic regulators of atherosclerosis progression. Further analysis regarding which specific gene within this cluster of three separate genes in the SLC22A3-LPAL2-LPA locus is actually linked to MI susceptibility, might in turn give clues as to how a decline in CFUs contribute to disease. Similarly, genetic interrogation of these cells might be useful in determining whether these two isolation approaches are actually measuring similar activities be it endothelial progenitor activity or some additional property relevant for CVD. Part of this answer can presumably come from analyzing how well these two isolation protocols track in healthy or disease populations. Here the record is mixed. For instance, in some studies where investigators have simultaneously executed both strategies the two approaches positively correlate with each other and both provide independent measurements of risk [4]. In contrast, other studies have suggested that in a given population, one method yields a tighter correlation with disease risk [8]. Finally, it has also been noted that in some populations, neither assay has been shown to be especially helpful in delineating risk [17]. In this context, a genetic determination of loci that determine abundance should be helpful in identifying whether cells isolated by flow cytometry and colony formation are biologically similar or distinct. For instance, further analysis of genetic factors determining the abundance of $CD34^+$ $AC133^+KDR^+$ cells can presumably be performed and compared to those factors regulating CFUs number that were described in the current study. Whether the loci identified overlap or are non-concordant will be instructive.

In summary, the manuscript by Shaw and colleagues opens up a new genetic-based approach to a well-studied but still frustratingly ambiguous area of investigation. The sheer number of previous studies linking various circulating cells to CVD disease suggests that a biologically important relationship might exist. Yet, to move beyond interesting correlations it is essential to begin to understand why the number of given cell type varies within a population and to understand how a decline or expansion of a certain cell type might contribute to a disease phenotype. The current manuscript provides a way to move forward and to hopefully fulfill the tantalizing promise of EPC biology and CVD risk.

References

1. Khot UN, Khot MB, Bajzer CT, Sapp SK, Ohman EM, Brener SJ, Ellis SG, Lincoff AM, Topol EJ. Prevalence of conventional risk factors in patients with coronary heart disease. JAMA. 2003; 290:898–904. [PubMed: 12928466]
2. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med. 2003; 348:593–600. [PubMed: 12584367]
3. Murphy C, Kanaganayagam GS, Jiang B, Chowienczyk PJ, Zbinden R, Saha M, Rahman S, Shah AM, Marber MS, Kearney MT. Vascular dysfunction and reduced circulating endothelial progenitor cells in young healthy uk south asian men. Arterioscler Thromb Vasc Biol. 2007; 27:936–942. [PubMed: 17255539]
4. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, Bohm M, Nickenig G. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med. 2005; 353:999–1007. [PubMed: 16148285]
5. Schmidt-Lucke C, Rossig L, Fichtlscherer S, Vasa M, Britten M, Kamper U, Dimmeler S, Zeiher AM. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: Proof of concept for the clinical importance of endogenous vascular repair. Circulation. 2005; 111:2981–2987. [PubMed: 15927972]
6. Richardson MR, Yoder MC. Endothelial progenitor cells: Quo vadis? J Mol Cell Cardiol. 2011; 50:266–272. [PubMed: 20673769]
7. Shaw SY, Blodgett DM, Ma MS, Westly EC, Clemons PA, Subramanian A, Schreiber SL. Disease allele-dependent small-molecule sensitivities in blood cells from monogenic diabetes. Proc Natl Acad Sci U S A. 2011; 108:492–497. [PubMed: 21183721]
8. Cheng S, Cohen KS, Shaw SY, Larson MG, Hwang SJ, McCabe EL, Martin RP, Klein RJ, Hashmi B, Hoffmann U, Fox CS, Vasan RS, O'Donnell CJ, Wang TJ. Association of colony-forming units with coronary artery and abdominal aortic calcification. Circulation. 2010; 122:1176–1182. [PubMed: 20823386]
9. Tregouet DA, Konig IR, Erdmann J, Munteanu A, Braund PS, Hall AS, Grosshennig A, Linsel-Nitschke P, Perret C, DeSuremain M, Meitinger T, Wright BJ, Preuss M, Balmforth AJ, Ball SG, Meisinger C, Germain C, Evans A, Arveiler D, Luc G, Ruidavets JB, Morrison C, van der Harst P, Schreiber S, Neureuther K, Schafer A, Bugert P, El Mokhtari NE, Schrezenmeir J, Stark K, Rubin D, Wichmann HE, Hengstenberg C, Ouwehand W, Ziegler A, Tiret L, Thompson JR, Cambien F, Schunkert H, Samani NJ. Genome-wide haplotype association study identifies the slc22a3-lpal2-lpa gene cluster as a risk locus for coronary artery disease. Nat Genet. 2009; 41:283–285. [PubMed: 19198611]
10. Patino WD, Mian OY, Kang JG, Matoba S, Bartlett LD, Holbrook B, Trout HH 3rd, Kozloff L, Hwang PM. Circulating transcriptome reveals markers of atherosclerosis. Proc Natl Acad Sci U S A. 2005; 102:3423–3428. [PubMed: 15728378]
11. Schlitt A, Heine GH, Blankenberg S, Espinola-Klein C, Dopheide JF, Bickel C, Lackner KJ, Iz M, Meyer J, Darius H, Rupprecht HJ. Cd14+cd16+ monocytes in coronary artery disease and their relationship to serum tnf-alpha levels. Thromb Haemost. 2004; 92:419–424. [PubMed: 15269840]
12. Rogacev KS, Ulrich C, Blomer L, Hornof F, Oster K, Ziegelin M, Cremers B, Grenner Y, Geisel J, Schlitt A, Kohler H, Fliser D, Girndt M, Heine GH. Monocyte heterogeneity in obesity and subclinical atherosclerosis. Eur Heart J. 2010; 31:369–376. [PubMed: 19687164]
13. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997; 275:964–967. [PubMed: 9020076]
14. Waterstrat A, Van Zant G. Effects of aging on hematopoietic stem and progenitor cells. Curr Opin Immunol. 2009; 21:408–413. [PubMed: 19500962]
15. Ito H, Miyake M, Nishitani E, Mori K, Hatano T, Okuda T, Konoshima T, Takasaki M, Kozuka M, Mukainaka T, Tokuda H, Nishino H, Yoshida T. Anti-tumor promoting activity of polyphenols from cowania mexicana and coleogyne ramosissima. Cancer Lett. 1999; 143:5–1. [PubMed: 10465331]

16. Leone AM, Valgimigli M, Giannico MB, Zaccone V, Perfetti M, D'Amario D, Rebuzzi AG, Crea F. From bone marrow to the arterial wall: The ongoing tale of endothelial progenitor cells. Eur Heart J. 2009; 30:890–899. [PubMed: 19299431]

17. Xiao Q, Kiechl S, Patel S, Oberhollenzer F, Weger S, Mayr A, Metzler B, Reindl M, Hu Y, Willeit J, Xu Q. Endothelial progenitor cells, cardiovascular risk factors, cytokine levels and atherosclerosis--results from a large population-based study. PLoS One. 2007; 2:e975. [PubMed: 17925881]