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Review

The genetics of exceptional longevity identifies new druggable targets for vascular protection and repair

Annibale A. Puca[a,b,1], Gaia Spinetti[a,1], Rosa Vono[a], Carmine Vecchione[b,c], Paolo Madeddu[d,*]

[a] IRCCS MultiMedica, Milan, Italy
[b] University of Salerno, Salerno, Italy
[c] IRCCS Neuromed, Pozzilli, Italy
[d] University of Bristol, UK

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ABSTRACT

Therapeutic angiogenesis is a relatively new medical strategy in the field of cardiovascular diseases. The underpinning concept is that angiogenic growth factors or proangiogenic cells could be exploited therapeutically in cardiovascular patients to enhance native revascularization responses to an ischemic insult, thereby accelerating tissue healing. The initial enthusiasm generated by preclinical studies has been tempered by the modest success of clinical trials assessing therapeutic angiogenesis. Similarly, proangiogenic cell therapy has so far not maintained the original promises. Intriguingly, the current trend is to consider regeneration as a prerogative of the youngest organism. Consequentially, the embryonic and foetal models are attracting much attention for clinical translation into corrective modalities in the adulthood. Scientists seem to undervalue the lesson from Mother Nature, *e.g.* all humans are born young but very few achieve the goal of an exceptional healthy longevity. Either natural experimentation is driven by a supreme intelligence or stochastic phenomena, one has to accept the evidence that healthy longevity is the fruit of an evolutionary process lasting million years. It is therefore extremely likely that results of this natural experimentation are more reliable and translatable than the intensive, but very short human investigation on mechanisms governing repair and regeneration. With this preamble in mind, here we propose to shift the focus from the very beginning to the very end of human life and thus capture the secret of prolonged health span to improve well-being in the adulthood.

Contents

Abbreviations: BPIFB4, bactericidal/permeability-increasing fold-containing family B member; CVD, cardio vascular disease; CHD, coronary heart disease; MI, myocardial infarction; HF, heart failure; LI, limb ischemia; AD, Alzheimer disease; AAV, adeno associated virus; APOE, apolipoprotein E; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase; VEGF-A, vascular endothelial growth factor-A; LLIs, long-living individuals; GC, genetic component; SNPs, single nucleotide polymorphisms; FOXO3A, forkhead box O3A; GWAS, genome-wide association studies; LAV, longevity associated variant; RV, rare variant; WT, wild type; SOD, superoxide dismutase; ER, endoplasmic reticulum; UPR, unfolding protein response; MNC, mononuclear cell; SDF-1α, stromal cell-derived factor 1α; MCP-1, monocyte chemoattractant protein-1; BH4, tetrahydrobiopterin; BH2, dihydrobiopterin; RNS, nitric radical species; ROS, reactive oxygen species; HSPs, heat shock proteins; NO, nitric oxide; cGMP, cyclic guanosine monophosphate; PKB, protein kinase B; PKA, protein kinase A; HSP90, heat shock protein 90; L-NAME, N-nitro-L-arginine methyl ester; PAD, peripheral artery disease; CLI, critical limb ischemia; L-arg, L-arginine.

* Correspondence to: Bristol Heart Institute, School of Clinical Sciences, University of Bristol, Upper Maudlin Road, Bristol BS2 8HW, United Kingdom.
E-mail address: Paolo.Madeddu@bristol.ac.uk (P. Madeddu).
[1] These author contributed equally to this work.

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1. Introduction

There are an estimated 7 million people living with cardiovascular disease (CVD) in the UK and 160,000 people die each year because of CVD. Coronary heart disease (CHD) caused by the narrowing of arteries that feed the heart is the UK's single biggest killer, being responsible for ~73,000 deaths each year, an average of 200 people each day. Acute myocardial infarction (MI), which is caused by the occlusion of a coronary artery, represents the most harmful form of CHD. Most of the current treatments are palliative, *i.e.* they reduce symptoms associated with heart dysfunction, without providing a definitive repair. Consequently, CHD patients undergo a progressive decline in the pumping function of the heart that ultimately leads to heart failure (HF). Today, post-infarct HF is the leading cause of invalidity, hospitalization, and mortality in patients over 65. Limb ischemia (LI), is also caused by arterial occlusion and manifests as claudication, foot ulcers, and gangrene. Revascularization by angioplasty is often unfeasible or ineffective, hence many LI patients are left with no therapeutic option rather than foot amputation, which is associated with a yearly mortality rate >25% [1].

Regenerative medicine aims to provide a definitive treatment of ischemic complications by promoting endogenous mechanisms of repair and delivering supply-side boosts of cells, genes, or proteins. Unfortunately, both gene therapy and cell therapy failed to achieve the initial promises and researchers are now trying to understand the reasons for the unsuccessful translation of preclinical studies. In addition, there is a growing interest in untangling the mechanisms that allow an efficient regeneration during early stages of the life, but are attenuated with aging.

A novel way to pursue an effective regenerative product would be to start from a solid genetic understanding of the causes of disease and its predisposing factors. The most important risk factor for CVD is aging, and, recently, huge efforts have been done to halt the aging process in order to prevent cardiovascular complications. On the other hand, CVDs represent the most important cause of death for both middle-age and elderly people. For instance, a follow-up study on a population of 14345 individuals of 44 years old men showed that 300 out of the 914 death for all causes died for CVD in the follow-up of 11.4 years. Men who maintained or improved fitness had 30% and 40% lower risk of corresponding mortality, respectively [2]. This data could be interpreted in two different ways: 1) exercise training is the only possible way to reduce mortality; 2) among people analyzed, the ones genetically predisposed to live longer are biologically younger and thus have a better attitude to fitness that further contributes to the reduced mortality. The second key of interpretation is also in keeping with a fundamental shift in the regenerative medicine strategy. While most attention has been focused on correcting mechanistic targets responsible for failed regeneration, a more effective approach would be to determine and exploit the factors that allow some individuals to avoid CVD or cope better with CVD when it occurs. This is the case of the long-living individuals (LLIs), *i.e.* the small number of (1/5000 born) people that survive to be 100 years old, and their closest relatives and offspring, which have higher probability to live long and healthy [3,4]. Centenarians are either healthy or survivors of diseases of aging, such as diabetes, cancer, and CVD. Thus, studying the centenarians' genetic code could disentangle how these individuals delay aging and escape or experience attenuated forms of CVDs.

In this review, we will discuss mechanisms of regenerative angiogenesis instrumental to therapeutic revascularization of ischemic tissues. In particular, we will focus on the novel approach of interrogating the genetics of exceptional longevity to unravel regulators of vascular function that could be exploited in effective proangiogenic therapies.

2. Reparative angiogenesis for CVD

The knowledge of the different phases of the angiogenic process, the cells involved, and the factors released have been exploited to treat aging-related diseases in which angiogenesis is dysfunctional. The process of vessel remodeling in the adult occurs through sprouting of new vessels from existing ones. This process known as angiogenesis is guided by endothelial cells (ECs) that switch from a quiescent to an active state in the presence of proangiogenic factors as reviewed in [5]. During the process, specialized ECs known as tip ECs, following proangiogenic cues, move towards the zone that needs to be vascularized. The cells behind the tip are known as stalk ECs and form the body of the nascent vessel. In addition, $CD34^+$ cells derived from bone marrow (BM) and released in the circulatory system upon activation by physical or chemical stimuli, formerly known as endothelial progenitor cells (EPCs), also contribute to the process mostly *via* the paracrine activation of resident ECs [6].

The possibility to restore angiogenesis therapeutically was applied for the treatment of coronary artery disease (CAD) and peripheral vascular disease (PVD) especially through the administration of proangiogenic factors like VEGF-A [7]. Indeed vascular endothelial growth factor-A (VEGF-A) triggers angiogenesis and also improves vessel function through the activation of eNOS (with the consequent production of NO) and activation of prostacyclin (PGI_2) [8]. However, despite the success of preclinical studies in animals, clinical trials in humans showed poor or no efficacy. The same results were obtained using other angiogenic factors like FGF-2 [9] or other technologies like gene therapy methods [10]. Most of the negative outcomes are related to bias in the study design, but also to the fact that angiogenic factors activity is more complex than expected. For instance VEGF *per se* is not sufficient to maintain vessels stabilized for a long time. In addition, the VEGF dosage should be finely tuned to avoid aberrant or poor angiogenesis [11]. The modulation in angiogenesis through cell therapy was also tested using BM-derived cells. These studies demonstrated that transplantation or chemokine-induced mobilization of BM cells in patients with CAD (reviewed in [12]) or PAD [13] improve ischemic tissue functions although only modestly. However, BM cells are not homogeneous as they include hematopoietic ($CD45^+$) and so-called endothelial progenitors ($CD34^+$) as well as non-hematopoietic ($CD45^-$) cells. Therefore, better isolation protocols and more specific markers are needed. Furthermore, more efforts should be done to better understand their mechanisms of action, including physical or paracrine interactions with resident cells. In addition, it is necessary to consider that there are inter-individual differences in the level of these cell populations which are affected by aging especially when associated with diseases like diabetes, hypertension and others [14]. In this complex context, new tools to promote a controlled and physiologic angiogenesis are needed.