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Myocardial Therapeutic Angiogenesis: A Review of the State of Development and Future Obstacles

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Abstract

A significant percentage of patients have coronary artery disease that is too advanced or diffuse for percutaneous or surgical intervention. Therapeutic angiogenesis is a treatment modality to induce vessel formation being developed for patients with advanced coronary disease that is not amenable to currently available interventions. A number of approaches to induce coronary collateralization are being developed. These include gene, protein, cellular and miRNA modalities, with each having advantages and disadvantages. At this time, no modality has come forward as the single clear choice, and combination therapies may provide synergistic benefits. However, there have been a number of recent studies advancing our knowledge as to how we can refine pro-collateralizing treatments. In this review, we will examine some recent successes and future obstacles in the effort to bring therapeutic angiogenesis to patients.

Keywords

Therapeutic angiogenesis; Coronary artery disease; Gene therapy; Stem cell therapy; Growth factor therapy; Endothelial dysfunction; Endogenous inhibitors of angiogenesis

INTRODUCTION

Coronary artery disease (CAD) remains a major cause of morbidity and mortality in the developed world [1]. Despite advances in pharmacotherapy, catheter-based interventions,

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and surgical techniques, there are a significant number of patients who are not candidates for any type of coronary intervention [2]. However, angiogenesis, the formation of microvessels from existing vasculature by the proliferation and migration of endothelial cells, is currently being investigated as a therapy for patients with advanced CAD. In the adult, angiogenesis is an innate response to decreased myocardial oxygen delivery, and while angiogenesis does occur in the absence of ischemia, the focus of this review will be on ischemic vessel formation. Angiogenesis leading to formation of collateral vessels can result in smaller territories of ischemic myocardium and decreased severity of ischemic events [3–5].

It is clear that patients respond variably to chronic myocardial ischemia, though it is unclear why. Therapeutic angiogenesis has been studied extensively as a treatment option for patients with severe CAD and no viable revascularization options such as percutaneous coronary interventions (PCI) or coronary artery bypass grafting (CABG). The goal of therapeutic angiogenesis, like PCI and CABG, is to improve the symptoms of CAD by increasing perfusion to the ischemic myocardium.

Much research has been performed, with the goal of safely inducing local angiogenesis in the setting of chronic myocardial ischemia to eventually create perfusing collateral vessels. Several different methods for inducing collateral growth are being explored, including techniques at the gene, protein, post-transcriptional, and cellular level (Table 1). Various delivery systems are also being investigated to bring these treatments to the ischemic myocardium. These include the delivery of therapeutics by adenoviral vectors, stem cell homing, catheter-based techniques, and surgically-based systems.

A number of large animal studies utilizing many therapeutic modalities and delivery systems have successfully induced collateral vessel formation in the setting of chronic myocardial ischemia [6–8]. Many of these studies were performed using healthy, young animals without comorbid diseases, as it is obviously not always possible to precisely mimic the human situation. In fact, most of the treatments that were successful in animals did not translate well to humans in a number of disappointing clinical trials. In the United States, 32% of the adult population has hypertension, 16% has hyperlipidemia, and 10% has diabetes mellitus [1]. These chronic diseases and the drugs used to treat them may have an impact on native and therapeutic angiogenic potential.

In this paper we will review the process of angiogenesis and examine some of the recent advances and future hurdles in the area of therapeutic angiogenesis.

NATIVE ANGIOGENESIS

Coronary vasculogenesis, angiogenesis, and arteriogenesis are a spectrum of processes that lead to vessel formation. Vasculogenesis is the formation of new blood vessels *de novo*, and is controlled by stem cells called endothelial progenitor cells (EPCs) [9–11]. In contrast, angiogenesis is the formation of microvessels from the existing vascular network by proliferation and migration of endothelial cells, while arteriogenesis is the growth and maturation of blood vessels through formation of a smooth muscle layer [12]. Mature

vessels are most likely to contribute to increased perfusion of ischemic myocardium, can be seen grossly on angiography, and are the ultimate goal in therapeutic angiogenesis.

Vasculogenesis and angiogenesis can be initiated in the adult heart by a number of stimuli including hypoxia. In these two processes, a multitude of molecular signaling pathways work in concert to lead to coronary collateral development (Figure 1). Several of these signaling molecules represent targets for therapeutic angiogenesis including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), nitric oxide (NO) and its precursors, Epidermal Growth Factor (EGF), stromal cell-derived factor-1 (SDF-1), Platelet Derived Growth Factor (PDGF), Placental Growth Factor (PIGF), and Transforming Growth Factor (TGF)- β , -1, -2, and -3 [13]. Many of the angiogenic pathways essential for vessel development in the embryonic period stagnate and are activated again during ischemia [14]. While this is a consistent and dependable process in embryonic cardiovascular development, it is less dependable in adults with ischemic heart disease. In a study by Meier, *et al.* [15]*, it was found that only one-third of patients with CAD developed angiographically significant collateral vasculature. For this reason, therapeutic angiogenesis is an attractive modality to treat chronic stable CAD. Therapeutic angiogenesis seeks to initiate the process that leads to the formation collateral vessels that may bypass stenotic or occluded arteries [16]. Potential therapies that exploit many of the known pathways for vasculogenesis and angiogenesis are being further developed and refined based on past successes and failures.

THERAPEUTIC ANGIOGENESIS

Gene Therapy

Gene therapy involves the delivery of genes to the myocardium resulting in the production of growth factors that will stimulate vessel growth. The growth factors (e.g. VEGF, PIGF, and FGF) are often the same used in protein therapy, but the advantage of gene therapy is the longevity of its effect [17]**. Exogenously delivered proteins and cells tend to have a short half life and thus a short amount of time to effect vessel growth [18]. Gene transfer therapy, on the other hand, can theoretically produce a more sustained effect due to prolonged expression by the host or viral machinery. The difficulty thus far has been in delivering an adequate amount of the target gene to the appropriate tissue. A number of different methods, such as the use of liposomes and viral vectors for gene delivery, are being used with varying success [19] (Table 2). Gene therapy has great potential for inducing angiogenesis, but like other therapies, has not transitioned well from the bench to the bedside [20].

Intravenous injection of naked plasmid DNA has been associated with a very good safety profile, but low transduction efficiency [21]. As the technology exists today, this is not the best method of delivery, and modalities that protect the DNA and improve transduction such as liposomes and viral vectors are being developed.

Liposomes are spherical nanoparticles composed of lipid bilayers and are being developed as vectors to deliver pro-angiogenic genes to the myocardium. The advantages of this delivery system are safety, large DNA capacity, the ability to direct the vector to the appropriate tissue type (i.e. tissue tropism), and traceability (usually based on fluorescent tags) [22]. Liposome and synthetic polymer carrier molecules have improved gene transfer

efficiency, but have the barriers of being immunogenic, inflammatory, and may be degraded by endosomes within the target cells [23]. More recently, viral vectors have been utilized, including retroviruses and adenoviruses [24,25]. This delivery option has the advantages of efficient delivery and tissue tropism.

Retroviruses deliver RNA which is integrated into the host genome, offering long-lasting effects. Retroviruses typically only transduce dividing cells which makes directing them to cardiomyocytes difficult, as these cells do not typically have a high rate of mitosis. Additionally, insertion of the gene into the wrong segment of DNA, or in the wrong cell type, can lead to insertional mutagenesis. This can potentially lead to the development of cancers such as leukemia or sarcoma [26]. Adenoviruses, on the other hand, can transfect non-dividing cells and do not integrate the transgene into the host genome, thus avoiding the problem of insertional mutagenesis. The advantages of using adeno-associated viruses is that they have a high rate of uptake in non-dividing cells, have a low immunogenic profile and have the ability to demonstrate tissue tropism. One disadvantage, however, is that they can cause an inflammatory response at high doses [27]. Recent studies have been geared toward adding constructs for more than one gene in viral vectors. In a recent study by Hu *et al.* [28] co-expression of angiopoietin-1 and VEGF led to smaller infarcts and improved post-myocardial infarction (MI) function than either gene alone in a mouse model of acute MI.

Unfortunately the ideal vector has not been found, and will likely need to be developed. It must avoid inducing an immune response, random insertional mutagenesis, and introducing wild type vector [29]. The ideal vector will also need to possess the following qualities: 1) non-toxic to cells, 2) efficient gene transfer to the host cell, 3) large capacity to hold genetic material, and 4) tissue tropism [30]. Currently the most common target genes for use in therapeutic angiogenesis are VEGF-A₁₆₅, FGF, transforming growth factor beta-1 (TGFβ-1), angiopoietin-1 and 2, and hypoxia inducible factor 1α (HIF-1α). These have been used in early clinical trials with varying results [31–35], and recently well reviewed elsewhere [36]. To further complicate the matter, recent publication of subgroup analysis from the AGENT 3 and 4 trials demonstrated a gender difference in patients receiving FGF gene therapy [37]. In this trial replication deficient, human adenovirus serotype 5 with human FGF-4 gene insert was delivered by intracoronary infusion in 532 patients with a history of recurrent stable angina. While there was no difference in Canadian Cardiovascular Society class in men, the women demonstrated improved total exercise tolerance test time, time to 1 mm ST-segment depression, time to angina, and Canadian Cardiovascular Society class. Future trials should consider possible gender differences.

Delivery of genes and vectors to the ischemic myocardium is another hurdle which needs to be overcome. Intramuscular, intravenous, catheter-based and surgical delivery methods are being investigated. Methods to deliver genes and vectors by catheter-based technology to the target site are among the most advanced at this time. Catheter-based delivery has been associated with low efficacy in the past, and techniques are being developed to improve delivery of genes and vectors. Naked DNA delivered and locally applied in polymer hydrogels by a catheter has been demonstrated [38]. One of the more viable and promising methods involves coating coronary stents with genes or vectors [29]. This is advantageous as stent technology is widely available. The stent is placed in close proximity to the ischemic

myocardium and remains in the vessel permanently. This allows prolonged exposure and release of the gene or vector to the endothelium to enhance the angiogenic effect [39,40]. However, delivery of drug and/or gene coated stents is an invasive procedure, even though other percutaneous, catheter-based, and surgical methods of intracoronary, intramyocardial, intrapericardial, and intraventricular injection are being utilized in clinical trials [36]. Ideally as vector tropism improves, less invasive delivery options such as intramuscular and intravenous injections can be used.

Growth factor therapy

The use of protein growth factors in therapeutic angiogenesis were the first modalities explored. The two most commonly studied growth factors are VEGF and FGF. VEGF-A is a mitogen that stimulates endothelial cell growth, and is a potent vasodilator secondary to production of nitric oxide (NO) and prostacyclin [41]. VEGF also stimulates endothelial progenitor cell (EPC) release from the bone marrow during hypoxia [42]*. It is this pleotropic influence imparted by growth factor therapy that makes it an attractive treatment modality. Initial studies in young and healthy animals were promising, demonstrating induction of angiogenesis in ischemic myocardium [6,8]. Unfortunately, like many other modalities of therapeutic angiogenesis use of VEGF and FGF in clinical trials did not yield robust results [35,43–45]. For example, the VIVA trial, a multi-center, double blind, placebo-controlled study examined 178 patients with coronary disease unsuitable for standard revascularization [44]. Patients were randomized to receive either placebo, low dose recombinant human VEGF (rhVEGF), or high dose rhVEGF by a single intracoronary infusion followed by multiple intravenous infusions. At 120 days there was no significant improvement in functional status as compared to the control group in the low dose group, while the high dose group did show significant improvement in angina class and favorable trends in ETT time and angina frequency. Other trials have presented similar findings of marginal or no improvement in functional capacity [46].

Before growth factor therapy can be a viable therapeutic option further research is needed. The reasons for a lack of effect of growth factor therapy and gene therapy may, in large part, relate to the fact that the recipient myocardium may not be receptive to the therapy. Growth factors such as FGF-2 and VEGF when used in porcine models of hypercholesterolemia produce little if any new vessel growth [47,48]. Models of type 1 diabetes and metabolic syndrome also have refractory mechanisms of collateral formation [49],[50]. Increased oxidative stress, seen in most comorbid diseases, is necessary for many cellular processes including angiogenesis, but at elevated levels may inhibit endogenous collateral vessel formation and inhibit pro-angiogenic effects of growth factors, genes encoding growth factors, and the engraftment of cells during cellular therapy [51]. In addition, the increased expression of endogenous inhibitors of angiogenesis (e.g. angiostatin and endostatin) and likely other anti-angiogenic factors are increased in patients with hypercholesterolemia and diabetes [52].

Cellular Therapy

Much research has been undertaken to more fully understand the reparative and regenerative potential of stem cells in chronic myocardial ischemia and after an ischemic myocardial

event. The advantage of this approach is not only delivery of cells that can replace damaged cardiomyocytes, but can secrete factors to support native cells leading to improvement in their function. Recent focus has been on exploitation of intrinsic progenitor cells and transplantation of specific cell types including: mesenchymal stem cells (MSCs), bone marrow derived progenitor cells, inducible pluripotent stem cells (iPS), and embryonic stem cells (ESCs). The success of cellular therapies will depend on whether the engrafted cells can differentiate into functional myocardial and vascular cells, and if these progenitor cells can produce paracrine signals that encourage survival of cells in the ischemic territory and surrounding normal myocardium [53]**.

MSCs have long been investigated for use in repairing damaged myocardium [54,55]. MSCs are multipotent cells that can be collected, cultured *ex vivo* to expand and differentiate the population, and subsequently delivered to the patient [56,57]. It has also been shown that they can be cryopreserved and successfully re-expanded for autologous use [58]. Transplanted MSCs have a low efficiency of engraftment *in vivo* and appear to yield a benefit only by expressing factors that support native angiogenesis and cell survival in the damaged myocardium [59]. Along this line, MSCs are now being modified to further support repair of the native ischemic myocardium. Survival proteins such as secreted frizzled-related protein 2 (sFRP2) [60] insulin-like growth factor 1 (IGF-1) [61] and the angiogenic protein angiopoietin-1 [62] are being overexpressed in MSCs and delivered to ischemic myocardium in small animal models.

Endothelial progenitor cells (EPCs), another type of stem cell, are a subset of bone marrow progenitors that may play a role in vasculogenesis either directly or as supporting cells [63,64]. It was initially thought that EPCs were only active embryonically, but it has become evident that they are also involved with endothelial repair and vasculogenesis in adults [65,66]. While circulating EPCs likely contribute to vasculogenesis, most interest is now in directed delivery of large numbers of cells to induce therapeutic vasculogenesis [67]. Early phase clinical trials have shown some benefit, improved exercise capacity and ventricular function in patients with ischemic heart disease [68,69]. However, like MSCs, there is controversy over the actual role of the transplanted cells in neovascularization [70]. It is now thought that EPCs, like MSCs, act in local signaling to enhance the endogenous repair mechanisms and angiogenesis [71]. As such, these cells have been shown to greatly enhance the angiogenic response to ischemia [72]. Further, like MSCs, efforts are being made to engineer EPCs *in vitro* to secrete higher levels of pro-angiogenic and pro-survival factors prior to transplantation [73,74]. Groups have created MSCs to overexpress PI3K/Akt and Bcl-2 to improve migration of stem cells and decrease apoptosis, respectively [75,76].

Inducible pluripotent stem cells (iPS) are yet another type of cell being explored as a therapeutic agent for CAD. These are somatic cells that have been re-programmed to function as pluripotent stem cells. Viral vectors are used to transfect genes to express factors such as Oct3/4, Sox2, Klf4, and c-Myc which return the fibroblasts and endothelial cells to embryonic-like stem cells [77]. Use of this cell type is still early in investigation and will likely require further refinement and testing to ensure effectiveness and safety prior to clinical studies [78,79].

ESCs represent a pluripotent stem cell type that may offer a wide variety of treatment options in CAD, from repair of damaged myocardium to whole organ regeneration. The use of ESCs in the United States has been controversial, and is likely in part responsible for the search and use of alternative stem cell types. However, ESCs have been shown to effectively differentiate into cardiomyocytes and other supporting cell types in a relatively efficient manner. In a study by Singla *et al.* [80] infarcted mouse hearts were injected with ESCs and demonstrated improved left ventricular systolic function and decreased myocardial remodeling. One of the side effects of ESC use is teratoma formation. Expression of TNF α has been shown to increase ESC differentiation into cardiomyocytes and decrease tumor formation [81]. Additionally, work is underway to further improve the efficiency of EPCs. Techniques such as differentiating EPCs into cardiomyocytes or endothelial cells *in vitro*, optimizing the timing of pathway activation during differentiation, and enriching the local *in vivo* environment to increase engraftment are being explored [82–84].

Recent clinical trials investigating the efficacy of cellular therapy for myocardial infarction have not produced particularly encouraging results [85–87]. The HEBE trial assessed the feasibility of intracoronary infusion of mononuclear bone marrow cells (MBMC) in patients with AMI [88]. While it randomized only 26 patients to therapy, there was a significant improvement in global left ventricular and regional myocardial function as well as decreased infarct size twelve months after therapy. However, results from the ASTAMI trial, a randomized placebo-controlled trial of 100 patients receiving intracoronary mononuclear bone marrow cells during PCI for acute MI, were recently published and showed different results. Three years after inclusion there was no difference in LV ejection fraction by echocardiography or magnetic resonance imaging [89]. Thus, there are many hurdles to be cleared before stem cells will be ready for use in clinical practice [90]. Isolated cells must be collected in sufficient numbers and with a high purity. Acquiring large numbers of cells may be difficult in the elderly and patients with metabolic diseases [91]. We must be sure cells are differentiated, or will differentiate, into the expected type. A recent review examined the status of the two major methods of converting cells to the desired type. These involve either *in vitro* directed differentiation, wherein pluripotent cells are stimulated by growth factors to become the desired cell type, or reprogramming, where one fully differentiated cell type is converted directly into another [92]. Directed differentiation is advantageous as it avoids the risk of immune rejection [93]. Safe delivery systems which bring the stem cells to the appropriate site and allow for proper spatial orientation need to be developed. Transplanted cardiomyocytes must appropriately couple with native cells to allow for transmission of electrical signals and allow the differentiated stem cells to contract in concert with the rest of the myocardium [94,95]. Along this line, arrhythmia has been shown to be a potential complication of stem cell use. In a follow up study of nine patients receiving skeletal myoblasts during CABG, left ventricular ejection fraction was significantly increased from baseline, but five patients required automated cardiac defibrillators for management of sustained/recurrent ventricular tachycardia [96]. In a recent review, a connection between the presence of inflammation, mode of stem cell delivery and the development of arrhythmias was presented [97]. It was described that patients are already at risk for arrhythmias due to ischemic heart disease and catheter based delivery may further induce local inflammation further increasing the likelihood of arrhythmias developing. Further, implanted cells may not

electromechanically integrate with the host tissue also leading to disrupted conduction. A study using a rat model of cell therapy postulated that downregulation of connexin43 results in altered intercellular communication leading to late-phase arrhythmogenicity [98]. In addition to the above challenges, providing a supportive environment for stem cell engraftment and survival is also necessary. This can be difficult as local milieu can be hostile during ischemia with ongoing inflammation and fibrosis, leading to increased apoptosis [99]. Dinsmore *et al.* reported that bone marrow derived myoblastic cells harvested from patients with ischemic cardiomyopathy injected into nude mice did not function as well as those taken from patients without myopathy [100]. Diabetes and dyslipidemia may also impair the potential of stem cells to proliferate, migrate, and engraft into myocardium. A recent study utilizing mice with streptozotocin-induced diabetes found decreased hematopoietic progenitor cells, a reduced repopulation capacity, and altered cytokine expression patterns in the bone marrow [101]. Finally, the host immune response must be circumvented for allogenic cells or any cells with foreign immunogenicity. This response may be stimulated by MHC mismatch, improper presentation of immunological signals, inadequate gene silencing, or aberrant antigen expression due to in vitro production [102]. As these problems are dealt with, cellular therapy may become a viable and effective therapeutic option for patients.

One interesting area of angiogenesis research is combining available treatment modalities for a synergistic effect [103]**. Growth factor therapy is now being used in combination with other therapies to further improve results. Positive results were shown in a study by Lu, *et al.* [104] using a combination of FGF and platelet derived growth factor (PDGF) in a chronic swine model of myocardial infarction. In this study, two growth factors simultaneously delivered to the ischemic myocardium resulted in increased blood flow, increased collateral density, and improved left ventricular ejection fraction. In another recent study, mesenchymal stem cells (MSCs) were transfected with genes to overexpress VEGF and/or stromal cell-derived factor 1 alpha (SDF-1 α) and injected into the myocardium of rats with acute myocardial infarction. Animals receiving the combination of SDF-1 α and VEGF overexpressing MSCs demonstrated smaller infarct size, improved left ventricular function and increased capillary density [105]. Other investigators are exploring different combinations of gene, cell and protein therapies to treat ischemic heart disease [106–108]. Delivery can be local (by catheter-based or surgical deposition) or systemic (intravenous or intraarterial). Surgical placement of pumps and/or controlled release beads offers the most sustained delivery system, and can improve longevity of effect [109]. However, growth factors, genes, or cells injected into the heart usually do not remain there for very long. Cells injected have been found to migrate to the lung, spleen and other organs in short time. Those cells that do remain usually die, and those cells that survive often do not function in concert with the native myocytes. Thus, technical problems such as appropriate delivery vehicles and nurturing the local myocardial environment are key issues that need to be resolved before any regenerative therapy will have much clinically relevant effect in patients.

MicroRNA (miRNA)

MicroRNAs have recently been described as regulators of gene expression [110,111]. They are short segments (20–25 nucleotides) of non-coding RNA that act at the post-

transcriptional level. miRNAs pair with complimentary mRNA and repress their translation [112]. It has been described as a “fine tuning” mechanism to control translation during rapidly changing cellular conditions [113]*. Although the exact mechanism of miRNA repression is not fully understood, it appears they are involved with almost all cellular processes and may influence a number of disease processes including heart disease and angiogenesis [114–117]. As their intrinsic function is being defined they are concurrently being investigated as a potential flexible and precise therapeutic agent. The enzymes that control the production and maturation of miRNAs are called Drosha and Dicer and if knocked-down lead to altered protein expression [118]. In a study by Fasanaro *et al.* [119] miR-210 was found to be upregulated during hypoxia and controls endothelial survival, migration and differentiation. Overexpression of miR-210 under normoxic conditions led to increased vessel formation in Matrigel. Conversely, silencing of miR-210 in hypoxic HUVEC cells resulted in decreased vessel formation despite the presence of exogenous VEGF. Thus, local control of miR-210 could offer a potential target for therapeutic angiogenesis. However at this time there are many aspects of miRNAs that are not well understood. It appears that miRNAs are cell specific, and can act as positive and negative mediators of protein expression depending on cell type. Additionally a single miRNA may affect many targets and an mRNA may be influenced by many miRNAs [120]**. For example, miR-221/222 in endothelial cells is involved in controlling pro-angiogenic protein endothelial nitric oxide synthase (eNOS), but in cancer cells has been found to control tumor suppressor p27 [121]. Therefore, miRNAs could be used to locally increase pro-angiogenic factors that effect angiogenic targets (e.g. VEGF, FGF, eNOS) or decrease anti-angiogenic factors (e.g. angiostatin, endostatin) to stimulate collateral vessel growth. As of now there are no clinical trials examining therapeutic angiogenesis through use of miRNA in patients with coronary artery disease. Prior to use of a particular miRNA a complete understanding of its effects in all tissues will be necessary to avoid unwanted side effects.

CONCLUSION

Ischemic heart disease and its complications are a public health problem throughout the developed world. Therapeutic angiogenesis has the potential to provide an option to patients with acquired coronary disease that is not amenable to current therapies. Great strides have been made in the understanding of gene, cellular and protein therapies, and further work is underway to transition these treatments to patients. While there are a number of promising therapeutic modalities there still remain a number of considerable obstacles that must be overcome. As this work is completed at the bench in animal models, there must be a concerted effort to find a role and integrate this knowledge into clinical practice.

EXPERT COMMENTARY

We are making great strides to develop better methods of myocardial repair and regeneration. A number of improvements in current modalities has been accomplished and new technologies are being developed. While many of these therapeutic modalities have the potential to be successful in humans, one of the most significant barriers may be elaborated by the patients themselves. Endogenous inhibitors of angiogenesis were originally described by Folkman [122,123]*. Angiostatin is a ~40-kDa cleavage fragment of plasminogen. It

inhibits endothelial cell migration and vascular tubule formation [124]. Endostatin, a 20-kDa product of collagen XVIII cleavage, interferes with VEGF and FGF signaling, induces apoptosis, and inhibits endothelial cell proliferation [125,126]. Over time a number of other inhibitors of angiogenesis such as arrestin, canstatin, and tumstatin have been described [127]**. In a recent study by our group swine with metabolic syndrome and given supplemental resveratrol were found to have collateral vessel densities that were not different from the animals with metabolic syndrome and no resveratrol, despite increased expression of VEGF and eNOS. Interestingly the level of tumor necrosis factor- α (TNF α) was significantly elevated as compared to normal diet and metabolic syndrome controls [128]. It has been shown by others that TNF α can act as an anti-angiogenic factor [129]. These anti-angiogenic signaling molecules are increased in metabolic diseases commonly associated with CAD and endothelial dysfunction such as dyslipidemia, diabetes mellitus, and hypertension [130].

Endothelial dysfunction and inhibitors of angiogenesis are obstacles to therapeutic angiogenesis that must be overcome [91]. Recent studies have demonstrated that endogenous inhibitors of angiogenesis negatively impact collateral development in ischemic myocardium in animals [49,131–133] and humans [52,134]. In a recent study by Sodha *et al.* [52] diabetic patients undergoing heart operations were found to have increased myocardial expression of angiostatin and endostatin, and these levels had significant negative linear correlations with coronary collateralization by Rentrop collateral score.

All modalities of therapeutic angiogenesis will need to overcome these endogenous barriers to collateral development in order to be successful in patients. As new therapies are developed there should be a goal to prove efficacy in animal models of endothelial dysfunction prior to trial in patients.

FIVE-YEAR VIEW

Therapeutic options for angiogenesis will likely improve over the next five years. Many investigators are working to move this field forward to bring therapeutic angiogenesis to patients. More advanced and refined gene therapies, along with improved delivery methods will be developed. Additionally, further investigation of combination therapies to induce collateral growth will surely be developed. It is clear that the process of angiogenesis is complex and requires local and systemic signaling to bring all the necessary components for repair and regeneration of damaged myocardium.

More attention will need to be focused on modulating chronic diseases associated with endothelial dysfunction and inhibitors of angiogenesis. Examination of new treatments such as resveratrol, high dose vitamin C and E, and existing medications such as HMG-CoA reductase inhibitors (statins) and thiazolidinediones (e.g. Rosiglitazone), need to be examined, not only for their effects on metabolic diseases, but also for their impact on myocardial angiogenesis [135,136]. High doses of both vitamin C and E have been found to lower levels of oxidative stress and may improve the altered microenvironment found in metabolic diseases to improve the myocardial response to therapeutic angiogenic agents [137,138]. Atorvastatin and other statin drugs are frequently prescribed to patients

with atherosclerosis and risk factors for coronary artery disease. In a recent study we hypothesized that atorvastatin would enhance endogenous angiogenesis or growth factor induced angiogenesis. While endothelial function was markedly improved in a porcine, high fat fed, chronic model of myocardial ischemia, atorvastatin actually diminished collateral formation [133]. Since plaque stabilization and a reduction of plaque vascularity may be a primary cause of the beneficial effects of statin therapy, the findings may in part explain why statins may simultaneously diminish collateral vessel formation and improve cardiovascular outcomes [139]. Resveratrol, a constituent of many red wines, has been proposed to improve cardiovascular health [140]. When resveratrol was given to pigs fed a high fat diet sufficient to produce the metabolic syndrome, collateral dependent perfusion was improved and regional left ventricular function was preserved [50]. Adjuvant therapies such as statins or resveratrol may play a role in improving myocardial perfusion, but a better understanding of the effects of the various drugs will be required, and an adjuvant drug that has a consistent beneficial effect will need to be discovered [141]. In summary, with a greater understanding of the endogenous processes and pleotropic effects of medications we will be in a better position to ensure success of clinical trials of pro-angiogenic treatments.

The field of molecular imaging is also advancing rapidly and will provide improved outcome measures of success in therapeutic angiogenesis [142–144]*. As spatial resolution improves, most imaging modalities (SPECT, PET, MRI, ultrasonography, echocardiography and CT) are being refined to provide visualization of collateral growth and development [145]. Currently, the FDA holds that the primary endpoint for approval of an angiogenic agent must be an improvement in exercise performance of treated patients. There is a strong and documented placebo effect in angiogenic trials, so development of robust and reliable methods of visualizing the process of vessel growth and reperfusion is important [146]. These developments will all be important in the ultimate goal of improving the lifestyle of patients with advanced coronary disease that cannot be addressed by other means.

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ABBREVIATIONS AND DRUGS

Atrovastatin	HMG CoA reductase inhibitor (statin)
CABG	coronary artery bypass graft
CAD	coronary artery disease
eNOS	endothelial nitric oxide synthase
EPC	endothelial progenitor cell
ESC	embryonic stem cell
FGF	fibroblast growth factor
iPS	inducible plueripotent stem cell

MSC	mesenchymal stem cell
NO	nitric oxide
PCI	percutaneous coronary intervention
PDGF	platelet derived growth factor
Resveratrol	a polyphenol found in high concentrations in red wine
TNFα	tumor necrosis factor alpha
VEGF	vascular endothelial growth factor

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Key Issues

- Coronary artery disease and its complications remain a leading cause of mortality in industrialized nations. The field of therapeutic angiogenesis seeks to induce growth of collateral blood vessels into ischemic myocardium. There has been limited success in patients.
- A number of approaches are being explored including gene, cell-based, and growth factor therapies. Combinations of different modalities for therapeutic angiogenesis are showing promise.
- Gene therapy offers the most prolonged effect, as target DNA can be integrated into the genome and express for a long period of time. Delivery to the appropriate tissue is a hurdle to implementation. Liposome and viral vectors are being utilized.
- Stem cell therapy includes a number of different progenitor cell types. Engrafted stem cells must differentiate into functional, cooperative myocardial and vascular cells, and/or produce paracrine signals that encourage survival of cells in the ischemic territory and the surrounding normal myocardium.
- Growth factor therapy involves local delivery of proteins involved with angiogenesis to the ischemic myocardium. Surgically placed pumps or time-released capsules allow for prolonged exposure of myocardium to growth factor.
- An obstacle all modalities of therapeutic angiogenesis must overcome is the endothelial dysfunction and endogenous inhibitors of angiogenesis seen in patients with metabolic diseases such as dyslipidemia, diabetes mellitus, and hypertension.

Figure 1. Molecular signaling involved with angiogenesis

This basic schematic shows some of the key enzymes and essential steps in collateral vessel formation. eNOS- endothelial nitric oxide synthase, FGF (R)- fibroblast growth factor (receptor), PDGF (R)- platelet derived growth factor (receptor), PLC γ -phospholipase C gamma, PKC- protein kinase C, TGF β (R)- transforming growth factor-beta (receptor), VEGF (R)- vascular endothelial growth factor (receptor), $\rightarrow$ enzyme activated, $\dashv$ enzyme inhibited.

Table 1

Advantages and disadvantages of available modalities for therapeutic angiogenesis.

	<u>Advantages</u>	<u>Disadvantages</u>
Gene	<ul style="list-style-type: none">• Longevity of effect• Tissue tropism	<ul style="list-style-type: none">• Low efficiency with available techniques• Safety• Delivery
miRNA	<ul style="list-style-type: none">• Offers “fine tuning” control over angiogenic process	<ul style="list-style-type: none">• Not well understood
Cellular	<ul style="list-style-type: none">• Could provide new cardiomyocytes• Provides support for regeneration and repair of damaged myocardium	<ul style="list-style-type: none">• Large numbers of cells required• Immunogenic• Delivery• Appropriate spatial orientation
Growth Factor	<ul style="list-style-type: none">• Relatively easy to produce	<ul style="list-style-type: none">• Short duration of effect (unless associated with implanted delivery device.• Side effects (e.g. local edema with VEGF)

Table 2

Delivery methods available for gene therapy.

	Advantages	Disadvantages
Naked DNA	<ul style="list-style-type: none">• Safe	<ul style="list-style-type: none">• Low transduction efficiency• Transient expression
Liposomes	<ul style="list-style-type: none">• Safe• Large capacity• Directable• Traceable• Activatable	<ul style="list-style-type: none">• Inflammatory• Sequestration by immune system• Degradation by endosomes• Low rate of transcription
Viruses	<ul style="list-style-type: none">• Efficient• Tissue tropism• Long effect	<ul style="list-style-type: none">• Immunogenic• Random insertional mutagenesis• Presence of wild-type vector