Extracellular matrix-derived peptides and myocardial repair

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Repairing cardiac tissue remains one of the most challenging goals in tissue engineering. Here, we discuss ways whereby we sought to treat myocardial infarctions using extracellular-matrix derived peptides. Using an ischemia/reperfusion myocardial infarction rodent model, we targeted these extracellular matrix-derived peptides to the myocardial infarct site and were able to induce angiogenesis and alter the negative remodeling seen after an acute myocardial infarction. Our results indicate a potentially new strategy for repairing damaged tissue.

Introduction

Myocardial infarction (MI) can lead to death and disability with a 5-year death rate for congestive heart failure of 50%.1,2 Currently, heart transplantation is the only successful treatment for end-stage heart failure. However, this treatment is limited by the availability of donor hearts.3,4 Thus, alternative therapies need to be devised to treat end-stage heart failure.

A viable vascular architecture to allow for cell survival, proliferation and differentiation is crucial to myocardial regeneration. The development of biocompatible polymers that can act as substrates for sustaining cell growth, differentiation and other biologically relevant functions has become an integral aspect of tissue engineering. The use of ECM-derived components is an area of active research in tissue engineering as a way to facilitate the regeneration or repair of damaged tissue in vivo. The ECM within the myocardium consists mainly of collagen (predominantly collagen Types I, III and IV), fibronectin, laminin and chondroitin sulfate.5,6 The cardiac ECM is not only responsible for maintaining myocardial resistance and tensile strength, but it also acts as a scaffold for storing and delivering cytokines, growth factors and hormones. As a result, the ECM plays a pivotal role in cell adhesion, cell signaling and cell proliferation, as well as in regulating cell and tissue organization and function. After MI, several remodeling events are induced.5 In the early stages, inflammatory and endogenous cells release metalloproteinases (MMPs), which contribute to the degradation of mainly collagen within the infarct zone. This loss leads to infarct expansion and progressive dilation of the left ventricle (LV). The heart attempts to heal itself by increasing the deposition of collagen types I and III. However this collagen is immature during the early healing process and is susceptible to stretch for several weeks. Hence, the LV is distensible during the first several weeks post-MI, making it susceptible to dilation and negative remodeling. The healing process of the MI depends on the extent of the negative remodeling, infarct size and healing conditions.

The goal for MI treatment is to prevent, limit and eventually reverse the negative structural remodeling that lead to LV dilation and impairment of LV function. We had shown that using the peptides alone was enough to induce neovascularization within the site of myocardial injury.7 We investigated whether functional moieties derived from the ECM proteins Col IV8-10 and fibronectin (FN)11-14 could be used to alter the microenvironment to favor neovascularization. Col IV is involved in promoting and regulating the formation,
elongation and stabilization of microvessels during angiogenesis. FN is involved in promoting wound healing by recruiting endothelial or epithelial cells. The ECM-derived functional moieties were chemically conjugated to a monoclonal antibody targeting an injury-specific antigen within the MI, which allowed us to intravenously administer the treatment.

Peptide Therapy for Treating Myocardial Infarction

Our lab had shown that the use of polymers derived from ECM proteins, i.e., fibrin glue, Type I Collagen and/or Matrigel, or even polymers that have been modified with ECM-derived functional moieties, can induce angiogenesis enhancing the formation of new capillaries and functional arterioles in the MI region, while at the same time induce a higher influx of myofibroblasts, which are known to help restore structural integrity to the infarcted scar tissue. However, one drawback to using these polymers is that they require direct injection or implantation to the injured site, which can result in further health complications. A less invasive procedure would be to somehow inject the polymer intravenously. Unfortunately, these polymers are too large to pass through the capillaries. One solution is either to use smaller fragments of the polymer or relevant functional groups derived from the ECM proteins, i.e., ECM-derived peptides. Although these ECM-derived peptides have been used to modify synthetic polymers to improve the materials’ ability to interact with the pre-existing tissue, no one has considered using these peptides alone to promote wound healing and tissue regeneration.

We postulated that it might be possible to use fragments or even peptides derived from ECM proteins rather than the whole protein itself to influence the microenvironment to promote regeneration of lost tissue within the MI. We had investigated four ECM-derived peptides—Hep I, Hep III, RGD and FC/HV. Our in vitro analysis of these ECM-peptides revealed that Hep I, Hep III and RGD were all able to promote cell attachment, proliferation and migration, although not necessarily to the same extent as their whole protein counterparts. With FC/HV, we noted adherent cells only within the first 24 h, followed by subsequent loss of these cells. Interestingly, all of our ECM peptides were able to induce Erk1/2 activation, which is involved in the signaling pathway leading to angiogenesis and arteriogenesis.

To test the in vivo effect of the ECM-peptides, we injected the peptides into rats two days after inducing a myocardial infarction. In order to target to ECM-peptides to the site of injury, we had conjugated the peptides to antibodies specific for an antigen expressed shortly after a myocardial infarction. This antibody also had the added advantage of maintaining the peptides within the injury site for longer periods of time. Nuclear distribution studies have found that the antibody remained at detectable levels for as long as two weeks. Histological assessment of the heart tissue harvested six weeks post-treatment indicated that Hep I, Hep III and RGD promoted angiogenesis and arteriogenesis within the injured heart tissue. Yet, despite their ability to promote angiogenesis, we failed to observe statistically significant improvement in both the size of the infarct scar and in heart function. At the same time, however, we did note that the Hep III-treated heart managed to prevent worsening of the heart function. Hep III peptides can interact with one another to form a polymer-like matrix. It is possible that a comparable situation is occurring in rats treated with Hep III and may explain why Hep III was the only peptide that was able to prevent further negative remodeling as indicated by the echocardiography data. Furthermore, the peptides do not necessarily have to be interacting with one another to form a matrix. They also can interact with surrounding ECM proteins in a similar manner to form a matrix, thus alter the structural properties of the infarct tissue within the LV and prevent the negative remodeling associated with a MI. The results with Hep III suggest that the formation of an in vivo scaffold changes the material properties of the LV to prevent LV aneurismal formation.

We have demonstrated that ECM-derived peptides can effectively help to produce an ECM favorable for angiogenesis. A critical barrier to tissue regeneration is the lack of an adequate vascular network. The creation of a vascular bed in the infarcted myocardium may allow for greater cell engraftment and survival. The use of the ECM-derived peptides alone, without growth factors or cells, was sufficient to promote an angiogenic response in infarcted rat hearts. The induction of new vessel formation suggests that targeting active components of the ECM can influence the microenvironment and allow the body to act as its own bioreactor to regenerate vital structures of the myocardium.

Future Directions

We have shown that the use of various ECM-derived functional moieties can be used in treating MI. Our target ECM-derived peptide therapy results present a new non-invasive strategy for regenerative therapies and a tool for investigating tissue repair and regeneration.

However, our current approach with our ECM-derived peptide therapy, whereby we injected the therapy only once one to two days post-MI, was not able to promote complete recovery of heart function and cardiac tissue. Recently, researchers have looked into incorporating their knowledge of the dynamic behavior of the ECM into their therapeutic designs. For example, epithelial cells do not survive in scaffolds designed from poly-L-lactic-acid or poly-lactic-co-glycolic acid even in the presence of growth factors, but they do survive on Matrigel, a reconstituted basement membrane that mimics many of the mechanical and biochemical properties of the ECM. One explanation may be that the epithelial cells can remodel Matrigel but not the synthetic scaffolds. These studies highlight the need for determining an efficient method for integrating the dynamic interactions between the cells and our ECM-peptide therapy to obtain successful tissue regeneration.

In addition, our functional studies reflect that the ability to promote neovascularization is not sufficient to promote complete cardiac regeneration after MI. Of the ECM-derived peptides we studied, Hep III was the only peptide that prevented further deterioration of heart function. Recall, our TEM images showed that Hep III could interact with itself, forming a
scaffold-like matrix. Our studies involving polymers have shown that the ability to provide structural stability within the infarct site is key to helping promote improvement in cardiac function, while reshaping the dilated LV reduce wall stress and improves LV function.\(^1\) 18,19,21,25 Our results with Hep III suggest that it would be interesting to identify or even design peptides that have the ability to interact with one another to form a matrix in vivo and to promote angiogenesis, thus addressing both the structural and vascularization requirements for myocardial regeneration after injury.

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