



Cardiac Biomarkers: a Focus on Cardiac Regeneration

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Abstract

Historically, biomarkers have been used in two major ways to maintain and improve better health status: first, for diagnostic purposes, and second, as specific targets to treat various diseases. A new era in treatment and even cure for the some diseases using reprogramming of somatic cells is about to be born. In this approach, scientists are successfully taking human skin cells (previously considered terminally-differentiated cells) and re-programming them into functional cardiac myocytes and other cell types in vitro. A cell reprogramming approach for treatment of cardiovascular diseases will revolutionize the field of medicine and significantly expand the human lifetime. Availability of a comprehensive catalogue for cardiac biomarkers is necessary for developing cell reprogramming modalities to treat cardiac diseases, as well as for determining the progress of reprogrammed cells as they become cardiac cells. In this review, we present a comprehensive survey of the cardiac biomarkers currently known.

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Two unique characteristics of the heart, among many less formidable ones, are responsible for the major challenges facing investigators when it comes to regenerating the heart. First, the heart functions in syncytium. In other words, newly-generated cardiac cells must learn to be in sync with resident cells—a function that necessitates the presence of intercalated discs, making possible electrical conduction that achieves a functional syncytium in a beating heart. Second, the heart is an exceptional organ in the sense that it spontaneously generates its own electrical impulses. Thus, a regenerated heart must possess its own pacemaker, a required component that initiates the heartbeat. These specialized cells not only spontaneously generate the impulse for heart contraction, but also dictate the initial rate of the beating heart.

All and all, one must appreciate that a variety of essential elements make cardiac cells unique among other types of

somatic cells. Cardiomyocytes compose a truly extraordinary organ, most notably, a structure which cannot be voluntarily or consciously controlled. The striated cells, assembled by the arrangement of protein filaments which interact to allow movement, also branch out and intertwine in a mesh-like formation with other cardiomyocytes. The formation of this tear-resistant weave is necessary due to the significant pressure the heart must withstand to efficiently pump blood throughout miles of vasculature within the body. Given the structural and functional complexities of the heart, the central question before us is: Can a specialized cell of this unique nature be created through reprogramming of somatic cells? In this review, we will attempt to answer this question in the context of internal and surface molecular markers, contractility, and synchrony of reprogrammed cardiac cells. Furthermore, it is noteworthy that impressive advancements

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in recent history demonstrate positive evolution toward engineering cells that function appropriately and satisfy the requisite characteristics of the functional cardiac tissue.

Intracellular Molecular Markers

Specific markers expressed by individual cells partially define their identity. A cell's membrane is particularly important to examine for the presence of identifying biomarkers. However, few cardiac muscle-specific surface indicators have been discovered, thus forcing researchers to utilize intracellular proteins extensively (if less efficiently) to identify cardiac muscle cells. Observations of intracellular molecular characteristics can reveal whether reprogrammed cells have differentiated into heart muscle cells or have remained what they were originally. The native cardiomyocyte cell has many key features, and notable vital proteins that serve as cardiomyocyte-specific biomarkers are examined here.

Transcription Factors

When verifying the differentiation of cardiomyocytes, it is important to observe the expression of transcription factor proteins. Transcription factors represent early cardiac markers and indicate cardiomyocyte progenitors. Nkx2.5, or cardiac homeobox protein, is a cardiac-specific gene and marker of cardiomyocyte differentiation. Nkx2.5 has been used regularly to demonstrate the differentiation of cardiomyocytes in one study by Chiavegato and associates¹ as well as by Kodama et al., in research of human CD 14+ monocytes (that incidentally also determined monocytes do have cardiomyocyte potential).² Tbx5, or T-box 5, is another transcription factor protein which has been connected with the developing heart. Along with signaling the development of the upper limbs, Tbx5 plays an important role in developing the cardiac septum as well as the electrical system that coordinates the contractions of the heart, and its presence would signify a heart muscle cell progenitor.³ One additional factor, Isl1, a LIM homeodomain transcription factor, has also been used to mark cardiac progenitors. Cai et al. found this protein to be vital to "the outflow tract, right ventricle, and much of the atria".^{4,5} While these three proteins will not be observed in all cardiomyocytes, their presence should not be disregarded when contemplating the muscle cells of the entire heart. In other words, their existence indicates the development of cells important to the entire heart organ.

Proteins GATA-4 and GATA-6 serve as further cardiac-specific marker genes. Both transcription factors are vital to proper cell function, as they are part of a network of transcription factors that interact closely to create working heart cells. Indeed, a number of gene knock-out studies have demonstrated the importance of these transcription

factors in shaping a functional heart. For example, Oka et al. revealed that the deletion of the gene for GATA4 could result in the failure of the fetal heart due to malformation.⁶ Further examination of the GATA-4 transcription factor in the adult heart also demonstrated its importance to the heart's ability to function properly under stress. In fact, when the GATA-4 gene was deleted in adult mice, it was observed that the mice lacking the transcription factor protein suffered deterioration of their myocardium, and the magnitude of the damage was particularly evident upon stress induced by exertion and exercise.⁶ Peterkin et al. found that GATA-6 was crucial for the development of the heart. Interestingly, both the contractile function of the heart and the expression of other regulatory genes (such as Nkx2.5 mentioned above) were impaired upon the absence of GATA-6, suggesting that GATA-6 was a master regulator of cardiac contractility.⁷

According to the findings in 2008 by the Leibniz Research Laboratories, murine-induced pluripotent (iPS) stem cells were successfully generated, and the reprogrammed cells nicely exhibited the cardiac features based on the analyses of molecular, structural, and functional elements. There were gene markers for mesoderm, cardiac mesoderm, and cardiomyocytes, which included, among many, such factors as GATA-binding protein, NK2 transcription factor, Brachyury, mesoderm posterior factor 1, atrial natriuretic factor, myosin light chain 2 atrial transcripts, myosin light chain 2 ventricular transcripts, α -myosin heavy chain, and cardiac troponin T. Cardiomyocyte proteins were also verified, such as sarcomeric α -actinin, titin and cardiac troponin T.⁸

Troponins

The mature myocardium of the heart consists of three main types of proteins: myofibrillar proteins, metabolic proteins, and extracellular proteins.⁹ Several myofibrillar proteins that are only expressed by the heart can serve as markers of cardiomyocytes. Two of these are the Troponin isoforms Cardiac Troponin I (cTnI) and Cardiac Troponin-T (cTnT). Though these regulatory myofibrillar proteins serve chiefly as biomarkers in a clinical setting for cardiac damage from myocardial infarction, some researchers have used them to show that cardiomyocyte differentiation has occurred. While concluding that whether or not stem cells had differentiated into cardiomyocytes remained "complex and only partly understood," Bin et al. used the presence of cTnT to indicate that cardiomyocytes had successfully differentiated.¹⁰ Wang et al. also used the appearance of cTnI, along with N-cadherin, to indicate that mesenchymal cells from the umbilical cord had differentiated into cardiomyocytes.¹¹ Saito et al. determined that bone marrow stromal cells implanted into the heart were capable of differentiation into cardiomyocytes by marking the expression of cTnI of the cells.¹² Ultimately, cardiac-specific troponins are important



since they function closely with the integral contractile proteins of the cardiomyocytes.

In an important 2009 study using human adult fibroblasts, Zwi et al. identified the molecular components of cardiomyocytes from human-induced pluripotent stem (hiPS) cells in their findings. One of their outcomes detailed that the gene expression for the hiPS cells demonstrated mesoderm and cardiomesoderm markers, cardiac-specific transcription factors, and structural genes. The pattern of the gene markers initially demonstrated a decrease in the pluripotent markers (OCT4, NANOG) along with an increase in the mesoderm and cardiomesoderm markers (Brachyury and MESP1), and the secondary heart field progenitor marker *Isl1* followed. The cardiac-specific structural genes developed, including sarcomeric proteins α -myosin heavy chain, β -myosin heavy chain, cTnI and ion channel proteins (MYH7, MYH6, cTNNI, MLC-2A) and ionic channels (CACNA1C, CACNA1D, hERG, HCN-2).¹³

Cardiomyocyte Surface Markers and Shape Characteristics

While internal cellular markers provide some clear evidence of cell differentiation and identification for researchers working with heart regeneration, one would wish to identify cell surface proteins that could be used to verify heart muscle cells. So far, these proteins have largely remained elusive. However, there are a couple of notable exceptions, along with the verified existence of intercalated discs in reprogrammed cells. Assuredly, much research continues in efforts to find additional tangible surface cell biomarkers.

Cell surface markers

Among the candidates for cell membrane markers are Popeye domain containing 2 (POPDC2) and potassium voltage-gated channel subfamily A member 6 (KCNA6).¹⁴ These surface markers have a high frequency of expression along with alpha-myosin heavy chain (MHC).¹⁴ POPDC2 has been seen in embryonic chick hearts.¹⁵ N-cadherin, a calcium-dependent transmembrane adhesion protein, additionally serves as an important protein marker of cardiomyocytes. Honda et al. found that embryonic stem cells expressing high levels of N-cadherin differentiated at a high rate into cardiomyocytes. Interestingly, these cells also exhibited “cardiogenic markers,” *Nkx2.5*, *Tbx5*, and *Isl1*.¹⁶

Examples of two surface cardiomyocyte proteins that have been identified are β_1 - and β_2 -adrenergic receptors. These proteins give cyclic adenosine monophosphate signals and normally reside in transverse tubules of the cell.¹⁷ Tomita et al. demonstrated that their differentiated cells exhibited β -adrenergic receptors, and the beat rate increased in response

to isoproterenol, a sympathetic beta adrenergic agonist. Given the sensitivity of the cardiac response to adrenergic hormones, i.e., epinephrine, it is logical to determine whether the presence of the adrenergic receptors defines the differentiation step to a functional heart. However, the question is how accurate of a cardiac-specific biomarker are the beta adrenergic receptors. This group also verified the existence of intercalated discs upon transplanting the cells into mouse left ventricles, resulting in cell survival and appropriate connection to resident cells.¹⁸

A unique feature of the heart organ is the presence of intercalated discs, which serve to support the synchronized contraction of the cardiac tissue. This makes the intercalated discs the perfect place to hunt for cardiac-specific biomarkers. Connexin 43 is an important cardiac surface protein for forming hemichannels and gap junctions within the heart on account of the fact that Connexin 43 is visible in the intercalated disks of the heart muscle.¹⁹ In addition to the intercalated discs verified by the Tomita group noted above, Yue et al. also managed to see Connexin 43 in cardiomyocytes differentiated from mouse embryonic stem cells. They determined that it was present in the cell-cell junctions and concluded: “Embryonic stem cell (ESC)-derived cardiomyocytes were coupled by gap junction in culture”, which is an important observation.²⁰

Remarkable developments in 2010 were documented by Zhang et al., who accomplished the formation of new cardiomyocytes without completely reversing the cells to a stem-cell state. In those results, researchers first produced cells that flattened and lost their striations, losing both their electrical phenotype and myofilament cardiac troponin T. Upon redifferentiation, the cells then exhibited α -MHC, appropriate cardiac gap junction protein Connexin43 and endothelial marker CD31. The methodology used by the Zhang et al. further uncovered some mechanisms underlying the special cell progression for cardiomyocytes, while revealing the possibility of using dedifferentiated adult myocytes to create cells with antigenic and morphologic features of cardiac stem cells.²¹ This study is innovative in the sense that it utilizes the power of specific biomarkers studied to assess the extent of the damage that can be inflicted on a heart before a point of no return is reached. In other words, we can envisage the development of a cardiac-specific biomarker panel to assist with the prognosis of the cardiac patients.

Cardiomyocyte shape

Cardiomyocytes do have a unique shape, and this shape has figured prominently in identifying this cell type for many years. Diez and Simm used flow cytometry to identify heart muscle cells based on their length (rod shape). These researchers also included some insight as to why it is difficult to discover cardiomyocyte surface proteins: These

cells are particularly susceptible to damage and have RNA that rapidly degrades, making them very difficult to study.²² Surface striations of cardiomyocytes are also one of their unique features, as was mentioned in several of the studies examined above. Gaustad et al. used binucleation and striation as the identifiers of the cardiomyocytes differentiated from the human adipose tissue stem cells that had been injected with the rat cardiomyocyte extract.²³ Research carried out by Zwi et al. on human iPS cells showed that the spatial orientations of cardiomyocytes within the beating areas were projecting in various directions. Their cells exhibited a striated pattern seen in those typical of early-stage myocytes, as well as additional indicators of gap junction development and electrophysiological coupling. Lastly, the team reported the human cell-derived iPS tissue responded to adrenergic and muscarinic signals. Upon isoproterenol administration, the frequency of beats increased, and conversely, a dose of carbamylcholine reduced the frequency of spontaneous beating. This last area presents significant implication for the use of induced tissue as a drug-testing platform; and indeed, further introduction of specific drugs affected the tissue similarly as compared to a regular heart.¹³

Surface characteristics and cell shape can be used to identify cardiomyocytes, in addition to several transcription factor genes. Cardiac cells can be identified by contractile myofibrillar proteins, some (and hopefully more to come) surface proteins, and striations along with shape and length. Many important studies have implemented these indicators as conclusive evidence of cells successfully reprogrammed into verifiable cardiomyocyte cells.

Contractility and Synchrony

The most dramatic and visible hallmark of the heart cell is its ability to spontaneously beat, which necessarily includes intracellular action potential communication. In many of the studies already cited, the ability of a cell to beat a rhythmic contractile phenotype was a key marker of its identity as a cardiomyocyte. Similar to other muscle cells, cardiomyocytes contain the contractile proteins actin and myosin. The cardiac-specific forms of these proteins, sarcomeric α -actin and sarcomeric myosin (and their associated proteins), are observed in functional cardiac muscle cells, and actin and myosin proteins have been used to identify cardiomyocytes in several studies of stem cell differentiation within the last decade.

As early as 2001, Kehat et al. determined that differentiated cardiomyocytes in vitro formed spontaneously-contracting areas and expressed cardiac myosin heavy chain, alpha actinin, desmin, cardiac troponin I, and ANP (atrial natriuretic peptide), and had myofibrillar organization upon inspection with electron microscopy.²⁴ Recently, Hossain et al. developed a method, through which they measured the degree and complexity of cardiac differentiation by analyzing

the force of muscular contractions following the exposure of cardiomyocytes to various positive or negative inotropic agents. In that study, three substances of caffeine hydrate, p-hydroxyphenylacetamide and calcium chloride dehydrate were used as positive, neutral, and negative inotropic agents, respectively. Given that cardiac synchrony is an absolute requirement for the proper pumping function of the heart, these findings suggested that we might someday develop a dedicated panel of the inotropic agents useful in quantitating the levels of cardiac synchrony.²⁵

In another example, Kumar et al. used sarcomeric myosin and α -actin as cardiomyocyte-specific markers in their study of transforming growth factor- β 2 (TGF- β 2) and embryonic stem cells. They were able to detect these proteins in the embryoid bodies after differentiation using TGF- β 2 and concluded that TGF- β 2 differentiated the embryonic stem cells into cardiomyocyte embryoid bodies, based partly on the fact that the embryoid bodies showed sarcomeric myosin and α -actin.²⁶ Recently, Vjoldani et al. used the microfilament protein alpha-actinin (as well as cTnT and myosin heavy chain) to identify cardiomyocytes that had differentiated from human lymphocytes.²⁷ That study suggested that cardiomyocytes might be potentially derived from the bone marrow myeloid lineage.

Research is abundant in which Myosin Heavy Chain and Myosin Light Chain were important cardiac markers. Bin et al. used Myosin Heavy chain as a protein marker,²⁸ and Hattan et al. used Myosin Light Chain as a marker when they purified cells differentiated from murine bone marrow cells.²⁹ Contractile myofibrillar proteins are possibly the most important markers for verifying that cells have differentiated into cardiomyocytes. Nearly every single study researched for this review included their presence as a noteworthy factor in the success of the experiment.

Examples of successful contractility after the process of reengineering include, among others, significant achievements by Takahashi and Yamanaka in 2006, as they reprogrammed mouse fibroblasts into cells characteristic of contracting mouse embryonic stem cells.³⁰ Additionally, notable accomplishment in reprogramming bone marrow mesenchymal stem cells for use as a transplantable bio-pacemaker was achieved by Tomita et al. in 2007. In their results, action potentials in the programmed cells initially began as sinus-node like signals but subsequently modulated into ventricular-type potentials. The research group also transplanted the cells into mouse left ventricles, resulting in cell survival and appropriate connection to resident cells with intercalated discs.¹⁸

Murine-induced pluripotent (iPS) stem cells grown by researchers at the Leibniz Research Laboratories in 2008 differentiated into cells that produced an average of 55% spontaneously-contracting cells, when compared against the well-established embryonic stem cells. The fluctuations of Ca^{2+} were similar to the embryonic cell derivatives, as was



the coupling of the cells. Additionally, electrophysiological analysis conducted revealed the expected response to β -adrenergic and muscarinic action potential signals.⁸

Noteworthy success manipulating murine cardiomyocytes was achieved by Pfannkuche et al., who closely studied the similarities between their iPS cell-derived cardiomyocytes compared to murine blastocyst-derived embryonic stem cells.³¹ They, too, generated beating cardiomyocytes from iPS-derived cardiomyocyte cells and were able to demonstrate significant similarity of cell-marker expression and intracellular organization of the structural proteins between the two cell types, as well as ventricular-type action potential signals, and tissues responsive to β -adrenergic influence. They concluded that induced cardiomyocytes should be considered as a potential source of cells for cellular cardiomyoplasty since they possess the requisite functional characteristics of a normal cardiac cell-spontaneous beating, hormonal regulation, cardiac ion channel expression, and contractility.³¹

In the study by Zwi et al., using human adult fibroblasts in 2009, the function, structure, and molecular components of cells from human-induced pluripotent stem (hiPS) cardiomyocytes were analyzed. Their results revealed several outcomes, including that the hiPS cell-based transduction method succeeded in producing cardiomyocytes in a similar differentiation process to the human embryonic stem cell system and that there was a resulting functional syncytium of cells, as opposed to isolated cells. Contractility was measured with a multi-electrode array recording system, which recorded total activation time and global conduction velocity, making possible the calculation of conduction velocity vectors. The local field potential duration was also measured, and the cell aggregates displayed rhythmic contracting as early as six days and continued strongly for several weeks. Notably, the syncytium demonstrated pacemaker stability and action potential generation in a coordinated manner.¹³

Scientists are becoming increasingly expert at cell manipulation and understanding at least some of the mechanisms behind cardiac functioning as shown by Zhang et al. in 2010, who purposefully dedifferentiated and then redifferentiated adult mammalian atrial and ventricular myocytes to form new cardiomyocytes without reversing the cells to a stem cell state. The cells in their study successfully exhibited rhythmic contractility along with the internal and external cell markers mentioned previously. An interesting speculation is made by the researchers, stating that perhaps nature performs spontaneous dedifferentiation as a survival mechanism in response to stressed cardiomyocytes - a hypothesis they alluded to in light of, among other indicators, the high existence of c-kit⁺ (a stem cell marker) in cells within failing human hearts.²¹

In another significant advancement, somatic postnatal and dermal fibroblasts were reprogrammed directly into cardiomyocytes by Ieda et al., in 2010, also bypassing an

induced pluripotent state. They were successful in achieving cells that contracted spontaneously and exhibited specific cardiomyocyte markers, including expression of gene character. This pioneering effort presented conclusive results showing that functional heart cells reprogrammed from somatic cells and that they could have potential use as a source for regenerative medicine.³²

On the heels of fibroblast reprogramming by Ieda and colleagues, compelling findings by Ding and associates at the Scripps Research Institute revealed that skin cells were directly reprogrammed to cardiac cells.³³ The researchers developed a method to bypass the complete regression to pluripotency by switching off the four inducing factors at an earlier stage (before pluripotency was reached). The signals to redifferentiate into cardiomyocytes were then introduced, and they discovered that they were able to use just three transduction factors (Oct4, Sox2 and Klf4). They recorded a "robust expression" of the cardiac indicators Flk1, Nkx2.5, and Gata-4 by day 9; and by day 11, there were beating cardiomyocytes, and markers were recordable for cardiac troponin T, sarcomeric myosin heavy chain, and α -actin. In addition, Connexin-43 was noticed along the periphery of many cells. "Forceful contractions" were noted by day 15, throughout complete samples. The immunocytochemistry analysis revealed heart cells of primarily the atrial subtype, based on the presence of the atrial isoform of myosin light chain (MLC-2a). They discovered in their process that the addition of cardio-inductive growth factor BMP4 augmented the contracting patches nearly 150-fold. The contraction frequency in their samples showed variation, ranging from 4 to 130 beats per minute, suggesting that some heart cells were acquiring "highly differentiated" attributes. Researchers were also able to observe proper calcium channeling with isoproterenol or carbachol for appropriate β -adrenergic and muscarinic signaling. Even action potentials upon electrophysiological assessment resembled those of the atrium, further underscoring the apparent atrial-like phenotype. The usual process for complete pluripotency inducement and redifferentiation can take up to four weeks, so the process formulated by Ding's group is significantly faster. In addition, their procedure reduces the tumorigenic issue that arises from regression to the complete pluripotent state.³³

Clinical and future focus

While crucial accomplishments exist that further the creation of cardiac-type tissue, including appropriate contractility, cell markers, and junction formation, clearly there is much left to discover and unlock in the field of reprogramming somatic cells toward cardiomyocytes and their direct clinical use. Yet, with each successive research effort, scientists are uncovering the inner molecular mechanisms that occur not only for cardiac specificity but

other cell lines as well. Among other issues left to analyze are transplant effectiveness and longevity for reprogrammed cells, while eliminating to the greatest degree possible any propensity for teratomas. Optimization of conditions and protocols must continue to be synthesized for continued adaptation toward the human system.

Cell reprogramming is simply turning back the biological clock of an existing differentiated cell in order to reprogram the cell with a new desired function. Biomarkers are the landmarks that can tell us at any given time which stage of differentiation a reprogrammed cell is undergoing. Given the potential of biomarker specificity, some investigators are currently focusing on utilizing the advances in identifying cardiac biomarkers (and closely related biomarkers) in individualized therapies for heart failure and fibrosis. Currently known biomarkers such as natriuretic peptides are not always the best indicators of heart failure and can be linked to other problems such as renal failure, making their diagnostic capabilities limited. Earlier this year, Januzzi et al. focused on fibrosis and cardiac remodeling and the role that novel biomarkers can play in their diagnosis. Alongside cardiac-specific biomarkers such as ST2 and troponin, they identified galectin-3, which is released by inflammation-seeking macrophages rather than the heart cells, as a critical marker of fibrosis after infarction since it induces fibroblasts to differentiate into myofibroblasts. In the context of clinical treatment, such biomarkers will be useful in creating personalized treatment plans for patients with heart failure. Monitoring the release of these biomarkers at various levels will allow the physician to identify which patients will need specific types of treatments.³⁴

While techniques for implanting need to improve before use becomes clinical practice, induced cardiomyocytes also signify a practical clinical advance in that they are patient-specific. Like the advances made in the discovery of novel biomarkers, these induced pluripotent stem cells will allow for continued development of treatment plans formulated specifically for the individual patient.

Another benefit of uncovering biomarkers for a reprogrammed cell is that it enables us to not only study the mechanism of the diseases but also to clinically monitor and follow the progression of the health or disease states of the induced pluripotent stem cells in their redirected course in patients.³⁵ Thus, while clinical applications of reprogrammed somatic cells currently remain limited, the implications of this research are great. Advances in this field will not only allow previously impossible treatments for human myocardial disease, but have already heightened awareness for the increased possibility of personalized treatment and therapies. A greater understanding of the individual's genetic cause of disease at the cellular level can give a greater effectiveness to the already existing treatments.

Apparent debate also exists within the current research community about the correct and best source for new cell

growth. Given the ability of the zebra fish to regenerate the heart tissue after having a section of its heart removed, researchers have been working intensely to unlock similar potential for human application, and some researchers currently believe that new heart tissue is created from mature myocytes, rather than stem cells.^{36, 37} Current research interpreted by Poss et al. and a second study by Jopling et al. indicate that a new zebra fish heart tissue is a result of the dedifferentiation of the current heart muscle fibroblasts, which then grow and divide to generate the replacement tissue.^{36, 37} It is this last step that must be particularly unlocked, as was indicated by Charles Murry of the University of Washington. He noted that muscle cells also dedifferentiate after injury in human hearts but do not go on to develop into new tissue for reasons that are as yet unknown.³⁸

Simultaneous research efforts by Joshua M. Hare and Louis Lemberg at the University of Miami,³⁹ however, have shown that the use of mesenchymal stem cells produces “dramatic improvements” in function and structure after heart attack. In animal studies of myocardial infarction, transplanted mesenchymal stem cells showed a resulting engraftment and differentiation into heart muscle cells and vasculature cells. Their research demonstrated that the scar size was minimized and the left ventricular function was increased.³⁹

Conclusion

Because of the unique anatomical and physiological features of the heart (intercalated discs, spontaneous nerve firing, and synchronicity in muscular contractions), identification of the markers specific to these unique characteristics may not only serve to track the ontogeny of a developing and regenerating heart but also assist in estimating the degree of heart damage. In this review, we summarized the scientific efforts aimed at identifying unique cardiac biomarkers with a focus on cardiac regeneration. As was described, most of these efforts have focused on the unique cardiac anatomical and functional features and used traditional methods to identify heart-specific biomarkers. Alternatively, with the rapid advances in large-scale screening approaches such as DNA microarray and proteomic techniques, we expect to see a significant surge in the number of new and unique cardiac biomarkers. In this regard, Gundry et al. are currently using proteomics in order to identify cardiomyocyte glycoproteins that can be used as the heart's identifiers.⁴⁰ With the vast amount of research in progress and significant ongoing discoveries, the ability to heal a damaged heart tissue with regenerated cells appears genuinely attainable.

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