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Systems-based technologies in profiling the stem cell molecular framework for cardioregenerative medicine

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Abstract

Over the last decade, advancements in stem cell biology have yielded a variety of sources for stem cell-based cardiovascular investigation. Stem cell behavior, whether to maintain its stable state of pluripotency or to prime toward the cardiovascular lineage is governed by a set of coordinated interactions between epigenetic, transcriptional, and translational mechanisms. The science of incorporating genes (genomics), RNA (transcriptomics), proteins (proteomics), and metabolites (metabolomics) data in a specific biological sample is known as systems biology. Integrating systems biology in progression with stem cell biologics can contribute to our knowledge of mechanisms that underlie pluripotency maintenance and guarantee fidelity of cardiac lineage specification. This review provides a brief summarization of OMICS-based strategies including transcriptomics, proteomics, and metabolomics used to understand stem cell fate and to outline molecular processes involved in heart development. Additionally, current efforts in cardioregeneration based on the “one-size-fits-all” principle limit the potential of individualized therapy in regenerative medicine. Here, we summarize recent studies that introduced systems biology into cardiovascular clinical outcomes analysis, allowing for predictive assessment for disease recurrence and patient-specific therapeutic response.

Keywords

Systems Biology; OMICS; Stem Cells; Cardiac Regeneration

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Stem Cells in the Post-Genomic Era

Stem cell biology has entered the post-genomic era, allowing for a holistic understanding of developmental molecular events through epigenetic, transcriptional, and post-transcriptional signaling. The science of integrating genes (genomics), RNA (transcriptomics), proteins (proteomics), and metabolites (metabolomics) data in a specific biological sample is known as systems biology [1]. Systems-based approaches utilize the combination of gene and protein expression array analyses, gene-gene and protein-protein interactions, and intracellular metabolite levels to understand simultaneous occurrence of molecular processes [2]. Deciphering critical molecular interactions through systems biology-based strategies could guide our goal to achieve functional and safe stem cell-based therapies.

Stem cells are characterized by an inherent ability to self-renew and differentiate into cell types from the three primary germ layers, providing a source for tissue regeneration. Stem cell behavior, whether to maintain its stable state of pluripotency or to prime toward a potential cell fate is governed by a set of coordinated interactions between epigenetic, transcriptional, and translational mechanisms. To fulfill the therapeutic potential retained in pluripotency, an understanding of molecular properties of self-renewal and commitment is required. Several investigators have addressed this challenge with systems biology approaches [3–6]. Specifically, Boyer et al used chromatin immunoprecipitation (ChIP) with DNA microarrays (Chips) also known as ChIP-Chip analysis [7] to identify the network of transcription factors that regulate stem cell fate [3]. Other studies have used *in vivo* biotinylation mediated ChIP (bioChIP) for global target mapping (bioChIP-Chip) and reported an expanded set of factors associated with pluripotency maintenance [4]. Compared to ChIP-Chip analysis, the bioChIP-Chip relies on streptavidin affinity capture of tagged proteins and circumvents issues related to antibody availability [8]. By combining this technique with whole-lane liquid chromatography–tandem mass spectrometry (LC–MS/MS), a commonly used method to measure nuclear protein levels, Wang et al studied the protein interaction network and identified factors with critical roles in stem cell pluripotency [5]. Systems-level analyses such as ChIP-chip and LC-MS/MS have been used to measure global change in histone acetylation and nuclear protein levels to understand stem cell fate change [6]. Similar studies assessed stem cell development on the basis of chromatin structure and its epigenetic modifications [9,10]. Indeed, integrating systems biology in progression with stem cell biologics can contribute to our knowledge of mechanisms that underlie pluripotency maintenance and guarantee fidelity of lineage specification.

Advancements in Stem Cell Biology

Natural and bioengineered stem cell populations have been identified including hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), tissue-specific adult stem cells, and induced pluripotent stem cells (iPSCs) (Figure 1).

- Human HSCs are found in the bone marrow, peripheral blood, and placenta, and give rise to all lineages of the blood [11]. Adult bone marrow-derived cells ($Lin^{-}CD34^{+/-}CD45^{+/-}c\text{-}kit^{+}$) have been shown to modestly augment cardiac function recovery by contributing to *de novo* myocardium in the post-infarcted

heart [12]. Alternatively, studies have used cell fate assays to report that HSCs do not transdifferentiate into cardiac myocytes in myocardial infarcts [13]. To better understand the molecular characterization of HSC microenvironments and the core genetic network responsible for HSC differentiation, systems-based approaches using messenger RNA (mRNA) and microRNA (miRNA) transcriptomes have determined a comprehensive list of hematopoietic regulators [14].

- Human MSCs are found in the bone marrow, adipose tissue, and the umbilical cord [15]. They have a propensity for multipotent differentiation into osteoblasts, chondrocytes, and adipocytes [16]. Bone marrow-derived MSCs were shown to be beneficial in the treatment of chronic ischemic cardiomyopathy [17,18]. Behfar et al primed bone marrow-derived MSCs with recombinant trophic factors including transforming growth factor-β (TGF-β) or bone morphogenetic protein (BMP), allowing for entry into the cardiac program [17]. Similarly, adipose tissue-derived MSCs, from minimally invasive liposuction [19,20], can transdifferentiate into cells with characteristics of cardiomyocytes and neovascular tissue [21]. However, recent studies observed a lack of spontaneous cell contraction in adipose MSC-derived cardiomyocytes [22]. Comparative analyses of MSCs from bone marrow, cartilage, and adipose tissue have been assessed for osteogenic, chrondrogenic, and adipogenic differentiation potential [23], yet it remains to be elucidated for cardiomyocyte differentiation. Indeed, advances in systems biology provide the tools to evaluate global molecular differences in MSCs due to variability in patient age, sex, and location of cell isolation. Recent studies utilized microarray technology for genomic profiling of bone marrow-derived MSCs and determined key molecules regulating stem cell survival, growth, and development [24]. Prior to harnessing their clinical benefit, the ability to track MSC regulatory pathway on a molecular level by transcriptomic, proteomic, and metabolomic analysis is required.
- Embryonic stem cells are derived from the inner cell mass of a blastocyst and are pluripotent, giving rise to endoderm, mesoderm, and ectoderm lineages [25]. Multiple mouse and human ESC lines have been established [26–28]. Beyond the unrestricted growth potential, ESCs create an immunological challenge for regenerative medicine [29], limiting therapeutic applications to preclinical studies. Systems biology approaches have been actively applied to study ESC properties including self-renewal maintenance and lineage commitment [30].
- The adult myocardium has a modest intrinsic regenerative capacity based on the presence of cardiac stem-progenitor cells [31–34]. This endogenous cardiac regeneration following injury is a highly debated event. Although some findings support the concept of the adult heart as a suitable target for regenerative intervention [35–37], the contribution of endogenous stem cells to restoring cardiac function is limited [38]. Despite the existence of c-kit+ population and the potential ability of bone marrow cells to facilitate cardiac repair, intrinsic mechanisms alone are inadequate to restore cardiac function to a failing heart [39]. Thus, strategies for guiding cells toward the cardiac lineage and stimulating proliferation of post-mitotic cardiomyocytes are needed. For this purpose, systems-based approaches

affords the ability to target genomic, proteomic, and metabolomic influences that direct *in vivo* and *in vitro* cardiomyocyte differentiation.

- Bioengineered stem cells, or induced pluripotent stem cells (iPSC), are generated by reprogramming somatic tissue, namely skin fibroblasts, using ectopic expression of defined factors [40,41]. Particularly, transcription factor sets Oct4, Sox2, c-Myc, and Klf4 or alternatively Oct4, Sox2, Nanog, and Lin28 are described to reprogram human somatic cells to the pluripotent state [42,43]. Studies show that iPSC-based transplantation into the adult infarcted heart modestly improves post-ischemic cardiac performance [44,45]. Despite the immunocompatible nature of iPSCs [46,47], their potential for clinical translation is currently hindered by the risk of dysregulated cell growth known as tumorigenicity [48]. Recent findings show that pharmacological purging with DNA-damaging agent, etoposide can reduce this threat of tumorigenicity [49]. While iPSC reprogramming event produces an embryonic stem cell (ESC)-like pluripotent state, human ESCs and their pluripotent transcriptome remain the standard for understanding lineage-specific differentiation [28,50]. Given the advantage of studying patient-specific and mutation-defined diseases using the iPSC platform, high throughput technologies and bioinformatics analyses could be progressively utilized to expand our knowledge on iPSC maturation potential and lineage specification.

Thus, a variety of sources for stem cell-based cardiovascular investigation are available. Prior to clinical-grade application, comprehensively mapping molecular signaling events that orchestrate different stages of stem cell differentiation into cardiac lineage is necessary. These molecular processes and regulatory mechanisms involved in heart development can be exploited to repair the injured heart and achieve tissue regeneration [51]. Additionally, the molecular and genetic composition of fully differentiated and functional cardiomyocytes needs to be defined. To meet these challenges, high-throughput technologies such as whole genome shotgun sequencing, deep sequencing, and CHiP-chip/CHiP-Seq are useful tools. Instead of focusing on the role of individual genes, proteins, and pathways in biological processes, the systems biology method allows for characterizing how molecular parts interact with each other to determine the collective intracellular dynamics as a whole [52]. Thus, the systems biology approach to understand commonalities of evolutionarily conserved regulatory networks provides new insight to controlling aspects of regeneration with transcriptomics, proteomics, and metabolomics.

Transcriptomics

Embryonic and adult stem cells possess a diverse developmental transcriptome, which is defined as the total set of RNA transcripts [53]. Recent findings suggest that stem cells vary in their differentiation potential towards highly specialized cells including cardiomyocytes [54]. Cardiogenesis is the developmental process of the embryonic heart and is tightly regulated by signaling pathways and networks of transcription factors [55]. Martinez-Fernandez et al demonstrate that transcriptome analysis can discern the maturation potential of bioengineered stem cells [54]. For instance, stage-specific cardiogenesis was assessed by genome-wide transcriptome analysis using distinctive mouse embryonic time points. Based on this referential gene expression guideline, it was determined that pluripotent cell lines

possess variant cardiogenic potentials depending on three-factor versus four-factor reprogramming method.

Systems-based approaches have been explored in similar studies to understand the cardiac developmental program. Faustino et al investigated the developing mesoderm transcriptome in mouse ESCs and identified over 8,000 genes underlying cardiac specification [56]. Incorporating this data with bioinformatics analysis streamlined upregulated and downregulated signaling components implicated in inducing the cardiac muscle fate [56]. Similarly, system-expression profiling, in conjunction with bioinformatics network analysis, allowed for identifying specific biomarkers (CXCR4/FLK-1) primed for cardiogenic specification [57]. Nelson et al used genome-wide microarrays on ESC-derived cardiac progenitors and identified a distinct transcriptome profile of 11,272 transcripts. Based on this information, bioinformatics dissection of exposed surface biomarkers prioritized CXCR4 chemokine receptor cluster as the most over-represented gene receptor family during pre-cardiac induction. Subsequently, CXCR+/FLK-1+ subpopulation was isolated from the ESC pool and differentiated to yield an enriched Mesp-1, GATA-4, and Tbx5 population, indicative of pre-cardiac mesoderm [57]. Indeed, identification of novel biomarkers and secreted cardio-inductive signals at each phase of myocyte differentiation can be achieved by genomic profiling of cardiogenesis [58,59].

Mapping transcriptome networks accelerates our understanding of stem cell-based cardiogenesis and its biological underpinnings. Embryonic heart formation occurs through strict combinations of extracellular signaling and structured patterns of timing that guide pluripotent cells through mesoderm induction into specialized cardiac cells, including atrial and ventricular cardiomyocytes, conducting cells, fibroblasts, and vascular endothelial and smooth muscle cells [60,51]. In the developing embryo, WNT and NODAL signaling networks are evolutionary conserved molecular cascades that induce mesoderm and endoderm, and are widely used to initiate cardiogenesis in ESC cultures [61,62]. Instructive guidance from NOTCH and FGF play an important role in the proliferation and fate selection of cardiac progenitors and is directed by a temporal window that is either inductive or inhibitive [63]. Global changes in the transcriptome of newborn cardiomyocytes revealed that myocardial NOTCH signaling drives cardiomyocytes to a conduction-prone phenotype [64].

Recent transcriptome studies identified factors that differentially regulate cardiogenesis from murine ESCs. Cai et al showed that both NODAL and TGFβ signaling induced early cardiac progenitor formation, yet NODAL expression declined due to feedback inhibition and TGFβ expression continued [65]. At later stages of cardiogenesis, TGFβ suppressed cardiomyocyte formation and stimulated vascular smooth muscle and endothelial differentiation [65]. Indeed, specification of cardiogenic cells is highly regulated by methodical exposure to these signaling factors. To comprehensively analyze the dynamic gene expression profile from the stem cell stage to adult cardiac structures, Li et al used a time-course transcriptome analysis of innate murine cardiogenesis [66]. Stage-specific analysis of the cardiogenic interactome identified developmental disturbances in epithelial-to-mesenchmyal transition (EMT), BMP signaling, NF-AT signaling, TGFβ-dependent EMT, and NOTCH signaling, elucidating regulatory networks at the boundaries of health and disease (87). Such initial

gene expression trends of cardiovascular development concur with the dynamism of lineage specification as each stage of cardiac differentiation assumes a discrete molecular fingerprint.

Identifying pro-cardiogenic genes by means of transcriptome analysis could allow for targeted execution of the cardiac program. High throughput approaches such as DNA microarrays have been used to elucidate cardiogenic instructive signaling in mouse ESCs [67]. Several studies agree that transcription factors including Nkx2.5, MEF2C, and GATA4 are upregulated with cardiac-specific commitment [56,68,67]. In addition, cellular binding small molecules including cytoskeletal, polysaccharide, and metal-ion-binding factors are upregulated during cardiovascular development [69,56]. Conversely, there is a decline of pluripotency markers including Oct4, Sox2, and Nanog, implying that differentiation downregulates components of DNA replication, cell cycling, and cancer mechanisms [56]. Gene Ontology Consortium studies of the differentiating transcriptome also found that downregulated transcripts exhibited significant RNA binding activity, ribosome structure, and translation regulators, defining a loss of oncogenicity associated with pluripotency and an acquisition of cardiac tissue-specificity [70]. This phenomenon of losing pluripotent expression prior to gaining mesoderm-specific markers suggests mutual exclusivity among each phase of cardiogenesis. Integration of gene expression profiles with proteomic and/or metabolic profiles could increase the precision of conserved cardiogenic transcripts.

Proteomics

Proteomics is defined as the large-scale study of protein abundance and its variations at specific time point [71]. Intracellular signaling events and post-translational modifications that direct self-renewal and differentiation events can be largely understood by mass-spectrometry-based proteomic technologies [72]. For example, 2D electrophoresis comparing the proteomic profile of spontaneous mouse ESC-derived cardiomyocytes and neonatal-derived cardiomyocytes yielded a 95% similarity of the proteins [73]. Similarly, human ESC-derived cardiomyocyte transcriptome revealed a gene expression profile at levels corresponding to 20-week fetal heart cells [74]. In contrast, Yin et al showed that ESC-derived smooth muscle cells (SMCs) expressed identical smooth muscle markers yet their proteome was vastly different from aortic SMCs [75]. This suggests that marker proteins used for characterizing mature cell populations may not be sufficient to classify stem cell-derived cell populations [75,76]. Discerning the divergent expression pattern between the naturally differentiated cell types and stem cell derived-mature cell types at both transcriptomic and proteomic levels could be considered an important step in advancing cell therapy-based regenerative medicine.

Proteomic methodologies used for lineage specification can reveal growth factor signaling relationships [77,78]. Although extracellular cues such as cytokines and matrix factors generate cell behavioral responses such as proliferation and differentiation, it is challenging to assign cue-response relationships in a direct manner [77]. Prudhomme et al used the computational partial-least-squares (PLS) analysis in combination with Western blot to delineate this cue-response relationship. Using this method, strongly correlated phosphorylated proteins involved in the signaling network that governs self-renewal versus

differentiation were identified [77]. Similarly, comparative proteomics by tandem mass spectrometry (MS/MS) revealed candidate effectors of mouse ESC cardiac differentiation [79], contributing to our understanding of guided developmental cardiogenesis.

Network hub analyses, a commonly used technique to map nodes and interactions of proteins, have cross-referenced subsets of stem cell-derived cardiac progenitors to reveal a robust pro-cardiogenic network *en route* to cardiomyocyte differentiation [80]. Targeted treatment with SDF-1, VEGF, and BMP2 was shown to activate signaling networks that guide cardiac determination [80]. Further network analysis of cardiogenesis-associated signaling cascades identified integrin, WNT/β-catenin, IL6, IGF-1 and cardiovascular hypoxia pathways as prominent in cardiac progenitor cells [56,81]. Such signaling networks converged with TGF-β, JAK/STAT, granulocyte-macrophage colony stimulating factor/colony stimulating factor 2 (GM-CSF/CSF2), and calcium signaling in stem cell-derived cardiomyocytes [82]. In addition to factors that accelerate cardiomyocyte differentiation, studies also described network decelerants that postpone cardiogenesis [56,83]. Identifying promoting and rate-limiting signaling hubs in the cardiopoietic framework could be advantageous in directing cardiac lineage specification.

Metabolomics

Integrating OMICS data in cardiovascular research could guide our understanding of global regulation of signal transduction and cellular metabolism [76]. In particular, the study of metabolomics captures the chemical fingerprint behind cellular processes. Bioengineered stem cells undergo a metabotype conversion from oxidative metabolism to glycolysis during nuclear reprogramming [84]. Conversely, during cardiogenesis, the metabolomic module acquires a mandatory switch from glycolysis to oxidative phosphorylation that drives ESC cardiac differentiation [85,86]. Chung et al assessed the glycolytic phosophotransfer network during mouse ESC cardiogenesis by high-throughput arrays [86]. Lactate-generating capacity of ESCs, quantified by uncoupling agents and respiratory chain inhibition, was measured during stem cell cardiac differentiation and correlated with array findings [86]. Linking alteration of cellular metabolism and function to both proteomics and transcriptomics could facilitate our goal in engineering specialized cardiovascular tissues.

Metabolomics allows for identification of unique biomarkers that are informative of cellular status during stem cell differentiation and dedifferentiation [87]. Specifically, intracellular (fingerprint) and extracellular (footprint) metabolomes have defined baseline stem cell metabolic landscape and its changes in the diseased state [88,89]. Studies have utilized metabolomic techniques to investigate the role of energy metabolism in controlling stem cell fate [90,91]. Mohyeldin et al showed that hypoxia-mediated activation of glycolytic metabolism increased the efficiency of nuclear reprogramming and maintained the pluripotent ground state [92]. Conversely, upregulation of the electron transport chain subunits and tri-carboxylic acid enzymes with decreased expression of glycolytic enzymes propagated cardiomyocyte differentiation [93,94]. Metabolic profiling techniques allow for the resolution of energy-dependent pathways that control lineage specification and could be utilized to enrich the examination of metabolic dynamics in different physiological and pathological states.

The main question arising from current studies in systems biology remains how these molecular regulatory mechanisms interact during cardiac differentiation. Investigating the transcriptional, translational, and epigenetic interplay would provide a holistic perspective in understanding cardiovascular development. One systems biology approach combined cardiac mRNA profiles with cardiac transcription factors (Gata4, Mef2a, Nkx2.5, and Srf), activating histone modifications and miRNA profiles [70]. In this study, Schlesinger et al showed that combinatorial regulation of mouse HL-1 cardiomyocytes exhibited interdependency among the three levels of molecular regulation, suggesting a capacity for mutual and reciprocal regulation [70]. Future studies directed at leveraging system interdependence may extrapolate novel therapeutic avenues in the context of regenerative cardiology.

OMICS Approach to Cardioregenerative Medicine

Introducing OMICS-based strategies into clinical outcomes analysis may allow for predictive assessment for disease recurrence and patient-specific therapeutic response. Recent studies using whole-genome expression microarray on blood samples from first-time acute myocardial infarction (AMI) patients elucidated differentially regulated genes and modulate pathways associated with recurrent cardiovascular outcomes [95]. Furthermore, corresponding bioinformatics analysis revealed increasing disease severity was associated with decreased expression of genes involved in the developmental epithelial-to-mesenchymal transition pathway, providing a cell-based approach for risk stratification in patients following AMI [95]. Disease anticipation prior to symptomatic presentation provides opportunities for proactive and preventative clinical management.

In the current era of cell therapy approaches for ischemic heart disease, clinical trials continue to evaluate the use of multipotent stem cells for cardiac regeneration. Investigators of the Phase I SCIPIO trial studied the delivery of mast/stem cell growth factor receptor Kit (SCFR; also known as c-Kit) positive cardiac stem cells and reported therapeutic benefit in LVEF of 12.3% ejection fraction units in the initial eight patients treated compared to baseline values [96]. Similarly, the Phase II C-CURE trial determined the safety of cardiopoietically-induced hMSCs in the context of heart failure and established a benefit in LVEF of 7% over baseline values [97]. Among the patients receiving C-CURE cell therapy that had 6-month follow-up (n=21) the reported change in LVEF varied from 2% to 10% over baseline values, suggesting a variability in patient response [97]. Genomic and proteomic analysis of patient-specific response could allow for stratification of therapeutic responders versus non-responders in cardiac regeneration (Figure 2). Additionally, current therapeutic benefit varies between clinical trials due to differences in transplanted cell types, differing in their paracrine potency, anti-apoptotic properties, tissue engraftment, and regenerative efficacy [98,99]. Such discrepancies could be analyzed using systems-based technologies to propose the most effective individualized cell therapy on a patient-specific basis.

Clinical Implications of Integrated Network Modeling

Novel insights into the biological networks governing cardiac regenerative mechanisms, including signaling factor and cytokine interaction, present innovative and effective strategies for applying stem cell therapeutics in acute regeneration of the infarcted ventricle [98]. Preclinical studies have validated the ability of cell therapy to increase myocardial functionality in various animal models, yet significant challenges preclude immediate clinical standardization of stem cell therapy [97]. The main challenges prior to achieving clinically meaningful regeneration include: (1) obtaining optimal amounts of primed cardiac progenitor cells and (2) assuring functional integration into the myocardium without inducing arrhythmic complications [35,100]. Systematic assessment of the signaling and genetic networks that target cardiac differentiation would advance our understanding of the genomic and proteomic regulators involved in enhancing cardiac repair. Utilizing OMICS-based network studies to standardize stem cell expansion and their cardiogenic guidance may facilitate implementation of safe and effective clinical translation (Figure 2).

The promise of cellular cardiogenesis and neovascularization using the regenerative platform necessitates thorough knowledge of the myocardial transcriptome, proteome, and metabolome. Genome-wide studies could elucidate key developmental circuits that might be involved in the transition of cardiac progenitor-stem cells into the cardiac muscle fate. Indeed, interdependent genomic and proteomic evaluation of adult stem cells demonstrating efficacy is necessary to devise an approach that maximizes cell capacity for repair prior to transplantation [101].

With the multiplicity of available stem and progenitor cell populations, the scope of clinical cardiac trials is continuously expanding [102,98]. Yet, a key concern in implementing stem cell transplantation therapy for heart disease is the selection of a particular developmental cell stage for post-engraftment safety and efficacy. Many studies have evaluated the generation and characterization of stem cell-derived cardiac progenitor cells, however little is known about the interdependent molecular signaling that subsequently converts committed cells into cardiomyocytes. Moreover, functional cardiomyocyte maturation including the acquisition of electrophysiological properties for conduction deserves further investigation. Understanding the cardiac transcriptome produces beneficial preclinical research regarding how each cell type affects cardiac performance and pathology. High-throughput systems biology approaches provide an unbiased holistic means to identify crucial signaling pathways involved in functional cardiomyocyte maturation and cardioprotective mechanisms. Integrated OMICS studies could yield global perspectives that view human development as a composition of highly interlinked cellular networks and bridge current knowledge gaps in myocardial lineage specification. Many investigators concur that a combination of tissue engineering, pharmacological approaches, and cellular transplantation, with appropriate quality control and validation of inter-trial consistency, would produce the safest and most promising therapies [103–105]. Indeed, system biology approaches characterizing the physiological and pathological cardiac blueprint can progress our ability to clinically regenerate the heart *in situ* and *in vivo.*

Conclusion

Current efforts in cardioregeneration based on the “one-size-fits-all” principle limits the potential of individualized therapy in regenerative medicine. Integrated systems biology presents the opportunity to match molecular defects in patients to the regenerative product required for safe and effective therapeutic benefit. Additionally, next generation systems biology approaches define with increased resolution the means for risk stratification in patients. In the era of translating stem cell advances into the clinic, bioinformatics serves as a critical tool to better inform our clinical practice and elevate patient care.

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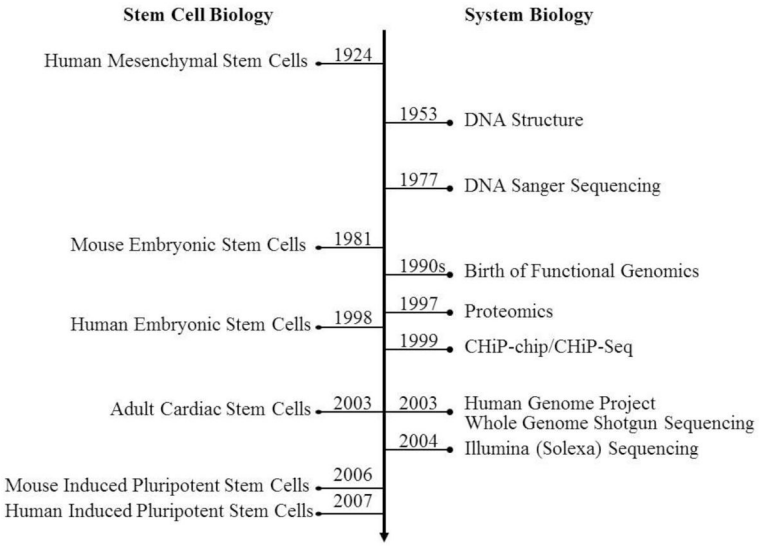

Figure 1. Initial developments in stem cell biology in parallel with the evolution of systems biology

Unraveling DNA structure, technological development of DNA sequencing, emergence of genomics, proteomics, and the completion of the genome project has coincided with critical discoveries in stem cell isolation and reprogramming strategies, resulting in cutting-edge efforts to utilize these findings for translational applications.

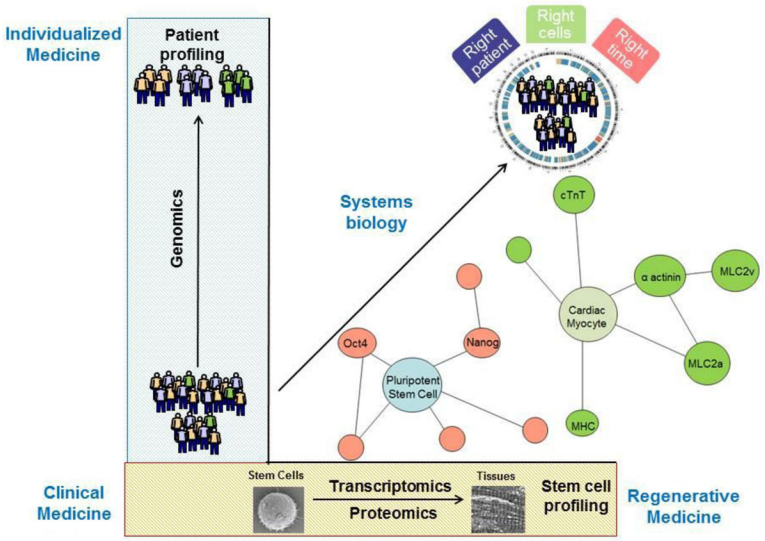

Figure 2. Integration of systems biology algorithms towards clinical application

Genomics, proteomics, metabolomics, and others allow for the elucidation of novel pathways involved in the step-wise differentiation of pluripotent stem cells to cardiomyocytes. Such bioinformatics analyses in the context of clinical translation can lead to patient-specific risk stratification and therapeutic response characterization.