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REVIEW

In vitro stem cell differentiation into cardiomyocytes Part 1. Culture medium and growth factors

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Summary Recent developments in the field of regenerative stem cell therapy for ischaemic heart disease have lead to an explosion in the clinical trial realm. At present no consensus exists regarding, amongst others, the optimal cell type as well as the underlying mechanism of action for any clinical improvement observed. As de novo reconstitution of myocardial tissue from multipotent stem cells is one of the working theories, the transdifferentiation potential of cellular populations under investigation into cardiomyocyte lineage phenotypes must ideally be assessed in preclinical bench work. Culture medium composition and a variety of growth factors are crucial determinants in this process. We discuss all relevant data acquired from in vitro work.

Contents

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Introduction

Heart disease was recognised as the most common cause of death in the western world as early as 1910 [1]. Congestive heart failure has become an increasing reason for hospital visits and more importantly hospitalization over the last decade [2,3]. Huge financial implications are also associated with this trend [3]. A fundamental shift in the aetiology of heart failure has taken place over the last decades. Hypertension and valvular heart disease have given place to coronary artery disease as the most common cause. In data analysed from 13 multicenter heart failure treatment trials, coronary artery disease was found to be the underlying aetiologic factor of heart failure in nearly 70% of the overall 20,000 enrolled patients [4]. Furthermore, the true prevalence of coronary artery disease among unselected heart failure patients is very likely to be underestimated due to the design of many of these studies [4].

Severe shortcomings are associated with current therapy of ischaemic heart disease, both in the acute as well as the chronic setting. In spite of advances in the treatment of acute myocardial infarction, including thrombolysis and percutaneous angioplasty/stenting, a considerable amount of irreversible myocardial injury occurs leading to adverse ventricular remodelling and myocardial dysfunction. Clinical concerns are even more obvious when dealing with established congestive heart failure of ischaemic aetiology. Once pharmacological treatment has been exhausted, clinicians do not have many viable options. More recently cardiac resynchronization therapy has been established as another palliative treatment but data on survival are still pending [5]. Surgical remodelling procedures are of limited application as the potentially superior effect of surgery compared to medical treatment alone remains to be shown [6]. The concept of an artificial mechanical heart remains remote with assist devices providing mainly a bridging solution at present. Despite the potential benefit of allotransplantation serious limitations apply to donor availability. The number of patients registered on the active transplant list on 31 March 2004 in the United Kingdom for a cardiothoracic transplant had decreased by 36% since 1995 [7].

Stem cell transplantation

Stem cell transplantation is being currently explored as a therapeutic approach to regenerate injured myocardium. Research is conducted at both the level of experimental animal and clinical human transplantation, with a variety of stem cell types being investigated [8]. Two stem cell populations appear to attract most interest owing to their degree of perceived plasticity. These include the totipotent/pluripotent embryonic stem cells and the multipotent adult stem cells.

The majority of the data accumulated to date originates from *in vivo* studies with plasticity of transplanted cells being attributed mainly to the mechanisms of milieu-dependent differentiation or cell to cell fusion. This may provide researchers with some optimism, but all underlying mechanisms continue to remain within the realm of medical hypothesis. The development of *in vitro* protocols to direct stem cell differentiation towards the cardiomyocyte lineage does not only assist in deciphering molecular signal pathways of differentiation, but also augments in creation of safer and more efficient stem cell transplantation models.

A plethora of molecular pathways is implicated in stem cell differentiation into functional myocardial cells once transplanted *in vivo*. Soluble chemokines, cell surface receptors, extracellular matrix substrata as well as intercellular gap junctions, are all potential cofactors. On the other hand as cell fusion has been shown to also take place [9] the molecular signalling pathways initiating this process have not been clarified. To be able to elucidate this process at a cellular and molecular level *in vitro* would translate to the ability to initiate and direct the differentiation process to a predefined point prior to clinical transplantation. Once the differentiation blueprints have been mapped out manipulation in a stepwise fashion will be feasible.

As expected by definition, stem cells demonstrate the inclination to undergo site-specific differentiation into multiple cell types once transplanted *in vivo* [10]. This may easily lead to suboptimal absolute cell counts regenerating the cell type of interest. In addition, a varying percentage of cells may be lost, e.g. due to local inflammation or because they do not 'home' to the site of injury. In order to overcome these issues it would be of major theoretical benefit to deliver pre-committed stem cell populations. Bittira et al. have actually shown that converting scar into myogenic tissue may be augmented by cell pre-programming before implantation [11].

Amongst the arguments opposing the use of undifferentiated embryonic stem cells in *in vivo* models is their potential to form teratomas once transplanted [12]. Although adult stem cells have not been associated with the development of tumour-like growths under routine *ex vivo* expansion conditions, prolonged *in vitro* culture of human mesenchymal stem cells has been linked with spontaneous malignant transformation [13]. This is of extreme importance if populations like the multipotent adult progenitor cells (MAPCs) [14] are to be considered for myocardial regeneration. Finally, Wang et al. demonstrated that bone marrow stromal cells can traffic through the coronary system to