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REVIEW

Stem cell integrins: Implications for ex-vivo culture and cellular therapies

Andrew B.J. Prowse*, Fenny Chong, Peter P. Gray, Trent P. Munro

The University of Queensland, Australian Institute for Bioengineering and Nanotechnology, St Lucia, QLD, 4072, Australia

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Abstract Use of stem cells, whether adult or embryonic for clinical applications to treat diseases such as Parkinson's, macular degeneration or Type I diabetes will require a homogenous population of mature, terminally differentiated cells. A current area of intense interest is the development of defined surfaces for stem cell derivation, maintenance, proliferation and subsequent differentiation, which are capable of replicating the complex cellular environment existing *in vivo*. During development many cellular cues result from integrin signalling induced by the local extracellular matrix. There are 24 known integrin heterodimers comprised of one of 18 α subunits and one of 8 β subunits and these have a diverse range of functions mediating cell-cell adhesion, growth factor receptor responses and intracellular signalling cascades for cell migration, differentiation, survival and proliferation. We discuss here a brief summary of defined conditions for human embryonic stem cell culture together with a description of integrin function and signalling pathways. The importance of integrin expression during development is highlighted as critical for lineage specific cell function and how consideration of the integrin expression profile should be made while differentiating stem cells for use in therapy. In addition this review summarises the known integrin expression profiles for human embryonic stem cells and 3 common adult stem cell types: mesenchymal, haematopoietic and neural. We then outline some of the possible technologies available for investigating cell-extracellular matrix interactions and subsequent integrin mediated cell responses.

Contents

Abbreviations: hESC, Human embryonic stem cells; MEK, mitogen activated protein kinase kinase; ERK, extracellular signal-regulated kinase; bFGF, basic fibroblast growth factor; IGF, insulin-like growth factor; ECM, extracellular matrix; VCAM, vascular cell adhesion molecule; ICAM, intracellular cell adhesion molecule; HSC, haematopoietic stem cell; mESC, mouse embryonic stem cell; MSC, mesenchymal stem cell; NSC, neural stem cell; ROCK, Rho-associated kinase; ICM, inner cell mass.

* Corresponding author. Fax: +61 7 3346 3973.
E-mail addresses: a.prowse@uq.edu.au (A.B.J. Prowse), f.chong@uq.edu.au (F. Chong), peter.gray@uq.edu.au (P.P. Gray), t.munro@uq.edu.au (T.P. Munro).

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Introduction

Human embryonic stem cells (hESC) were first derived in 1998 (Thomson et al., 1998) and in the short period since are beginning to realise their potential as a reproducible source of cells for clinical applications. In July this year, the United States Food and Drug Administration gave approval to Geron Corporation for a Phase I clinical trial to use hESCs for treatment of acute spinal cord injury (Briefing, 2010).

HESC culture and derivation conditions have trended toward the development of a fully defined, animal protein free system suitable for generating hESC or their functional differentiated progeny for clinical applications. Considerable research efforts have led to the identification of key signalling pathways for hESC maintenance such as the transforming growth factor-β superfamily [Activin, nodal, bone morphogenetic proteins, growth differentiation factors (Hannan et al., 2009; James et al., 2005; Vallier et al., 2005)], growth factors that activate receptor tyrosine kinases (e.g. mitogen activated protein kinase kinase [MEK] and extracellular signal-regulated kinase [ERK]) such as basic fibroblast growth factor (bFGF) and platelet derived growth factor (Amit et al., 2000; Pebay et al., 2005; Li et al., 2007; Kang et al., 2005), the insulin −like growth factor (IGF) and Wnt pathways (Sato et al., 2004).

As well as identifying soluble maintenance factors, there has been a large focus on the composition of the extracellular matrix (ECM) required for hESC growth (Xu et al., 2001; Amit et al., 2004; Evseenko et al., 2008). This has led to discovery of a number of ECM molecules or domains thereof capable of assisting in the maintenance of undifferentiated hESC alone or in combination, including laminin 511 (Miyazaki et al., 2008; Rodin et al., 2010), fibronectin and vitronectin (Braam et al., 2008; Rowland et al., 2009; Prowse et al., 2010; Melkoumian et al., 2010). However, little investigation into why or how each of these ECM molecules assists in maintaining hESCs has so far been conducted. ECM molecules such as the ones listed above are bound by cell surface proteins known as integrins. Evidence exists that removal of hESCs from their ECM substrate and passaging by dissociation into single cells is detrimental to their survival and differentiation capacity (Ungrin et al., 2008; Pera and Tam, 2010) initiating signalling cascades for anoikis (Wang et al., 2009). However, addition of inhibitors of the Rho-associated kinase (ROCK) signalling pathway can be used to improve survivability (Watanabe et al., 2007) but in single cell suspensions only a 58% survival rate is observed (Steiner et al., 2010). For hESC up-scale for therapeutic applications these methods are not a viable option. More recently, large scale screens of small molecule libraries have identified compounds that improve hESC survival, the actions of which have been linked to cell-cell and cell-ECM interactions through cell surface molecules such as integrins and cadherins that block ROCK pathways (Xu et al., 2010). Specifically, the MEK-ERK signalling cascade has been shown to be activated in hESC to promote self-renewal and survivability (Armstrong et al., 2006; Brill et al., 2009) both by extracellular growth factor addition (Li et al., 2007; Kang et al., 2005) and through activation of cell surface integrins (Xu et al., 2010). Examples in the literature have demonstrated the changing ECM and integrin profile of hESC as they differentiate (Wong et al., 2010; van Laake et al., 2010) although it appears the changes may be independent of growth conditions for undifferentiated cells (Braam et al., 2008). This indicates that in order to generate mature, functional and viable differentiated progeny from hESC for clinical applications the pathways by which ECM molecules signal to the cells needs to be better understood in addition to those already investigated for soluble maintenance factors [for review see (Pera and Tam, 2010)]. Furthermore, ECM receptors such as integrins can activate nearby growth factor receptors such as fibroblast growth factor receptor, epidermal growth factor receptor 1, insulin-like growth factor receptor and subsequent downstream survival pathways such as mitogen activated protein kinase and ERK (Comoglio et al., 2003). In this review we discuss the basics of cell surface integrin expression and examine the changes and importance of integrins during development using the mouse as a model. We then compare integrin expression profiles from hESC and other human adult stem cell types and conclude by reviewing studies that have used defined surfaces and high throughput technologies that could be used in order to better understand hESC-ECM interactions and integrin mediated signals. Due to length constraints on the review article the authors apologise for not highlighting all relevant references.

Integrins

Integrins are a large family of receptors which can bind ECM components, soluble extracellular ligands or other membrane bound cell surface molecules. In mammals, there are 24 known heterodimeric integrin receptors comprising of a non-covalent pairing of one of 8 β subunits with one of 18 α subunits. The different heterodimers each have specific ECM binding partners