Stem Cell Research (2012) 8, 97–108

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Laminin-511 expression is associated with the functionality of feeder cells in human embryonic stem cell culture

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Received 7 January 2011; received in revised form 19 August 2011; accepted 22 August 2011
Available online 27 August 2011

Abstract Fibroblast feeder cells play an important role in supporting the derivation and long term culture of undifferentiated, pluripotent human embryonic stem cells (hESCs). The feeder cells secrete various growth factors and extracellular matrix (ECM) proteins into extracellular milieu. However, the roles of the feeder cell-secreted factors are largely unclear. Animal feeder cells and use of animal serum also make current feeder cell culture conditions unsuitable for derivation of clinical grade hESCs. We established xeno-free feeder cell lines using human serum (HS) and studied their function in hESC culture. While human foreskin fibroblast (hFF) feeder cells were clearly hESC supportive, none of the established xeno-free human dermal fibroblast (hDF) feeder cells were able to maintain undifferentiated hESC growth. The two fibroblast types were compared for their ECM protein synthesis, integrin receptor expression profiles and key growth factor secretion. We show that hESC supportive feeder cells produce laminin-511 and express laminin-binding integrins α3ß1, α6ß1 and α7ß1. These results indicate specific laminin isoforms and integrins in maintenance of hESC pluripotency in feeder-dependent cultures. In addition, several genes with a known or possible role for hESC pluripotency were differentially expressed in distinct feeder cells.
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Abbreviations: hESC, human embryonic stem cell; ECM, extracellular matrix; HS, human serum; hFF, human foreskin fibroblast; hDF, human dermal fibroblast; mEF, mouse embryonic fibroblast; FBS, fetal bovine serum; GMP, good manufacturing practice; CM, conditioned medium; hiPSC, human induced pluripotent stem cell; FGF, fibroblast growth factor; TGFß, transforming growth factor beta; ActA, activin A; KAL1, Kallmann syndrome 1 sequence; mESC, mouse embryonic stem cell.

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doi:10.1016/j.scr.2011.08.005

1. Introduction

Undifferentiated human embryonic stem cells (hESCs) are traditionally cultured on mouse embryonic fibroblast (mEF) feeder cells in either fetal bovine serum (FBS) or serum replacement supplemented culture medium (Thomson et al., 1998; Draper et al., 2004). The exposure of hESCs to reagents of animal origin is likely to contaminate the cells with animal pathogens and non-human, immunogenic molecules (Martin et al., 2005; Heiskanen et al., 2007). Future clinical use of stem cell based products requires defined, Good Manufacturing Practice (GMP) compatible culture systems that are free of animal-derived reagents.

There have been two main approaches for replacing mEFs: the use of human feeder cells and the development of feeder-independent culture conditions. The feeder-independent culture systems often rely on the use of Matrigel extracellular matrix (ECM) preparation and mEF conditioned hESC medium (CM) (Xu et al., 2001). More defined culture methods based on human basement membrane protein coatings and serum-free culture media have been described (Ludwig et al., 2006a). Many of the feeder-independent culture conditions, however, are not defined and have been difficult to reproduce with different hESC lines (Hakala et al., 2009; Akopian et al., 2010). The derivation of new hESC and human induced pluripotent stem cell (hiPSC) lines is still routinely performed on supportive feeder layers, although there are a few recent studies describing hESC and hiPSC lines established on ECM preparations (Ludwig et al., 2006a; Klimanskaya et al., 2005; Fletcher et al., 2006; Lagarkova et al., 2010; Sun et al., 2009).

Feeder cells play a crucial role in maintaining undifferentiated hESC morphology and pluripotent status in feeder-dependent culture conditions. Human fibroblasts originating from different sources show varying capacity in supporting undifferentiated hESC culture. Clearly, the most supportive fibroblasts are derived from fetal tissue (Richards et al., 2003; Genbacev et al., 2005; Chavez et al., 2008; Kumar et al., 2009; Kibschull et al., 2011). Ethical considerations in using aborted human fetuses make this tissue source an unattractive option. We have derived and successfully propagated eight Regea hESC lines using commercial human foreskin fibroblast (hFF) (CRL-2429, ATCC) feeder cells (Skottman, 2010; Rajala et al., 2010). The hFFs have become the most commonly used human feeder cell type for hESC derivation and culture (Hovatta et al., 2003; Inzunza et al., 2005; Aguilar-Gallardo et al., 2010; Strom et al., 2010), and even human dermal fibroblasts (hDF) have been used for hESC propagation (Richards et al., 2003; Tecirlioglu et al., 2010). Skin is an easily accessible tissue source for derivation of fibroblasts, but there are notable differences between the capacities of different fibroblast lines to support hESC cultures (Eiselleova et al., 2008). Reasons for this are poorly understood.

The fibroblast feeder cells support hESCs by secreting growth factors and ECM components to the culture medium and also by directly interacting with the hESCs through cell–cell contacts. The factors secreted by fibroblast feeder cells have been studied by analyzing fibroblast CM (Lim and Bodnar, 2002; Prowse et al., 2005; Buhr et al., 2007; Chin et al., 2007; Prowse et al., 2007) and de-cellularized matrices with mass spectrometry (Abraham et al., 2010). Several ECM proteins such as collagens, fibronectin, laminins, nidogen and heparan sulfate proteoglycans have been suggested as key factors provided by the hESC-supportive feeder cells (Lim and Bodnar, 2002; Prowse et al., 2005, 2007; Abraham et al., 2010). Vitronectin (Braam et al., 2008; Prowse et al., 2011), fibronectin (Amit and Itskovitz-Eldor, 2006), laminin (Xu et al., 2001; Rodin et al., 2010) and a combination of collagen IV, vitronectin, fibronectin, and laminin (Ludwig et al., 2006a), have been used as substrata in feeder-independent culture of hESCs, showing that these ECM proteins, in combinations with specific media and growth factors, support attachment and proliferation of hESCs.

In addition to ECM proteins, growth factors provided by the feeder cells or in the culture medium are essential for hESC growth. The central role of basic fibroblast growth factor (bFGF) in hESC self-renewal is well established (Dvorak et al., 2005; Levenstein et al., 2006). Basic FGF and transforming growth factor beta (TGFß) family members TGFß, activin A (ActA) and nodal (Vallier et al., 2005; Xiao et al., 2006) co-operate to maintain undifferentiated hESC growth. The gene expression and secretion of these key growth factors have been shown to differ between hESC-supportive and non-supportive feeder cells (Eiselleova et al., 2008; Kueh et al., 2006).

In this study, we have compared the hESC-supporting capacity of in-house derived, hDF feeder cells to the commercial hFF feeder cells in a xeno-free culture system, based on the use of human serum (HS)-containing culture medium. We further studied the differences in ECM gene expression and synthesis and secretion of key growth factors between these fibroblasts. We found significant differences in the capacity of the different fibroblast cell lines to support hESC self-renewal and in the production of distinct candidate proteins such as laminin-511.

2. Results

2.1. Derivation and culture of xeno-free human dermal fibroblasts

Our initial goal was to derive xeno-free hDF feeder cells that could be used for the production of GMP-grade, clinical quality hESCs. A total of 6 pediatric skin tissue pieces were donated in context of surgery and 5 cell lines were successfully derived from the tissues. The methods were extensively optimized to find simple, effective and eventually GMP-compatible derivation, culture and cryobanking procedures. Explant culture was found superior to enzymatic digestion of dermis with collagenase, due to simplicity and low cost. HS concentrations from 5% to 20% were tested and 15% HS was found adequate for optimal growth. Heat inactivation (30 min at 56 °C) of HS substantially decreased growth rate and quality of the culture. The outgrowth of fibroblasts was commonly detected within a week. The fibroblast growth typically slowed down after 6–7 passages. All five hDF lines were successfully recovered after cryopreservation.

When used as hESC feeder cells, none of the five hDF lines could support the undifferentiated state of hESCs. Our routinely used, supportive hFF CRL-2429 line was transferred to identical HS-based culture system for comparison. On hFF CRL-2429 the hESCs retained their undifferentiated morphology (data not shown) confirming that hESC differentiation was not caused by the xeno-free feeder cell culture conditions,

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