Synthetic peptide surfaces for maintenance and expansion of human embryonic stem cells

Rohini Gupta*, Lauren E. Little*, David V. Schaffer, Kevin E. Healy.
University of California, Berkeley.

Statement of Purpose: Current feeder-free culture systems for maintaining self–renewal of human embryonic stem cells (hESC) use either animal or human derived extracellular matrix (ECM) proteins to coat the culture substrata; the most common of which is Matrigel, a mouse tumor cell derived ECM. For clinical applications, it is desirable to eliminate animal components from hESC culture systems. Integrin engagement particularly through the $\alpha_6\beta_1$ integrin is important for maintainaing hESC attachment and self-renewal(1). An alternate to using animal derived ECM is to employ short synthetic petides that mimic the appropriate integrin engagement domains and promote hESC adhesion and self-renewal. These synthetic peptides can be used as a biomimetic surface for hESC culture. To identify novel peptide sequences that bind hESC, a unique bacterial ligand display technique was used. This technique has recently been shown to successfully identify peptides that support neural stem cells (2). In this work, we evaluated whether novel peptides were capable of supporting hESC for multiple passages in vitro. We evaluated cell attachment, proliferation and pluripotency of two hESC cell lines in two medium conditions.

Methods: Bacterial peptide display technology as previously described (2) was used to identify peptides that A) bind with high efficiency (>50%) to hESC or B) target the $\alpha_6\beta_1$ integrin and bind hESC with high efficiency (>50%). For Group B, a negative selection process was first applied to identify bacterial clones that did not bind to hESC whose α_6 and β_1 integrins were blocked. These clones were then positively selected for their capacity to bind unblocked hESC (i.e. target the α_6 and β_1 integrins). Peptide 15-48+ (LEQRVGREMHSKWKR) from Group A and peptides 15-36 +/- (FKKRDRDKPPHRKYM), Co-34+/- (NQKEGEKVWYVVRRF) from group B were identified as strong biomimetic candidates. Two other peptides, AG-73 (CGGRKRLQVQLSIRT) and bsp-RGD(15) (CGGNGEPRGDTYRAY), were also used. For all adsorption studies, peptides were dissolved in synthesis grade water and adsorbed onto tissue culture polystryene (TCPS) at 200 μm concentration for 3 hours at room temperature and stored at 4°C until use. Prior to cell plating, surfaces were rinsed with PBS. HSF-6 (UCSF) were maintained in X-vivo-10 (Lonza) medium supplemented with 80 ng/mL FGF-2 (Peprotech) and 0.5 ng/mL TGF-β1 (R&D Systems) and H1s (Wicell) were maintained in mTESR™1 complete (Stem cell Technologies) medium. hESCs were cultured on Matrigel control or peptide surfaces. Cells were fed daily and passaged 1:2-1:3 every 5 days by exposure to Collagenase IV and scraping. Cells were visualized with phase or fluorescent microscopy.

Results: HSF-6 cells in X-vivo medium initially attached on the three selected peptide surfaces at similar density ($7x10^4$ cells/cm^2) as the Matrigel controls and were significantly higher than the TCPS negative control. Similarly after 5 days, HSF-6 on the three target peptides had high proliferation rates that were similar to Matrigel control plates (not significantly different). Next, we evaluated long-term culture hESCs (H1 cell line) in mTESR medium on peptide surfaces. Initial studies indicate that hESC attach and remain pluripotent on all three peptides for 5 days similar to Matrigel (Fig. 2).

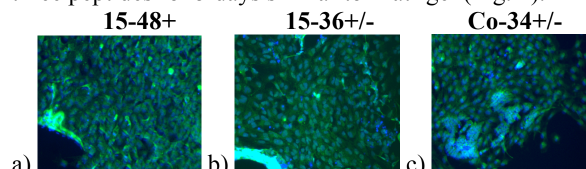

Figure 2: Oct-4 expression (green) overlaid with Dapi (nuclei, blue) of H1 on peptide surfaces a)15-48+ b)15-36+/- and c) Co-34+/- after 5 days.

Our previous studies have shown that hESCs use several integrin subunits (α_6, β_1, $\alpha_2\beta_1$, and $\alpha_v\beta_3$) for adhesion and proliferation (1). Thus, we devised a combination surface that consists of three peptides: i) 15-48+, Co-34+/- or 15-36 +/- peptides that engage with α_6 and β_1 integrins; ii) bsp-RGD(15) peptide that engages with $\alpha_V\beta_3$ integrin; and, iii) AG-73 peptide that binds with surface syndecan-1. A triple peptide combination with a 60:16:24 molar ratio successfully maintained hESC attachment over 5 passages similar to Matrigel controls (Fig. 3). Future work will evaluate hESC pluripotency on these unique triple peptide surfaces.

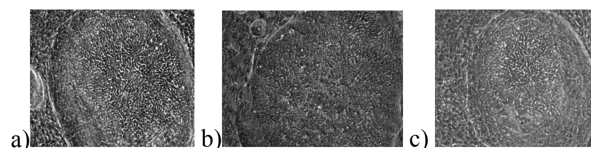

Figure 3. Phase images of H1 Passage 5, day 5 on triple peptide combination surfaces with target peptide a)15-48+ b)15-36+/- and c) Co-34+/-.

Conclusions: We have demonstrated that bacterial display technology can successfully identify novel peptides that support hESC culture *in vitro*. Two different hESC lines (HSF-6 and H1) attach, proliferate and remain pluripotent on these peptide surfaces when cultured in chemically-defined media. Moreover, when the peptides are added in combination with two other cell surface engaging peptides, hESC can be cultured on these surfaces for multiple passages.

References: 1. Meng, Y. FASEB J, 2010. 24(4): p. 1056-65. 2. Little, L. Chem Rev, 2008. 108(5): p. 1787-96

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