High Throughput Genomic-Guided Biomaterials Development for Regenerative Medicine Applications

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Statement of Purpose: Development of biomaterials for regenerative medicine applications has included approaches to mimic the extracellular matrix (ECM). There are hundreds of different ECM components within cell and tissue environments. Moreover, stem cells require specific ECM for their needs in different developmental stages. Identification of appropriate ECM surrogates for cellular differentiation and growth is a challenge for biomaterials development because little is known about most of the ECM proteome. To address the problem, effective methodologies to screen a wide range of biomaterials candidates for stem cell differentiation applications are needed. We have recently developed a novel high throughput ECM screening method which is guided by the genomic analysis of cellular differentiation. By investigating gene expression of stem cells during differentiation, we were able to mine from these data highly up-regulated ECM-related genes. We hypothesize that expression of these genes is a controlling factor in assembling the ECM that mediates stem cell differentiation. Further, that the genomically-identified ECM molecules can be implemented into novel biomaterials that modulate stem cell growth and differentiation.

Methods: To prove the principle, human amniotic fluid derived stem cells (hAFSC) were used in this study. Microarrays were performed on hAFSCs at various timepoints following myogenic, hepatogenic, vasculogenic, & osteogenic differentiations. RNA was isolated using RNAseB and hybridized to the Affymetrix U133A GeneChip. Highly up-regulated genes involved in ECM formation were identified through a customized microarray analysis algorithm. To demonstrate these target ECM molecules could serve as potential biomaterials targets, several were selected for further study under osteogenic differentiation based on their significantly up-regulated genes, availability and cost. ECM coatings containing a range of target molecules with different concentrations and combinations were prepared on standard cultureware by overnight incubation. hAFSCs were cultured on these coatings for up to 25 days in osteogenic differentiation media. Early or late osteogenic differentiation was evaluated by the production of alkaline phosphatase and bone mineralization (alizarin red assay), respectively.

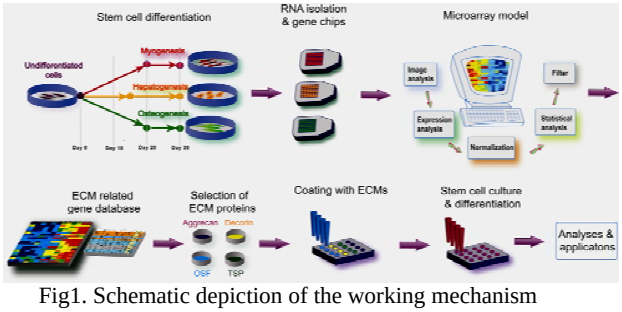

Fig1. Schematic depiction of the working mechanism

Results/Discussion: 1. ECM-related gene expression upon cellular differentiation. Microarray analyses of differentiating hAFSCs provided more than 40 ECM-related genes that were universally up-regulated as these cells reached their terminal fate. Some of the most significantly up-regulated genes are those involved in ECM production. As shown in Table 1, four ECM-related genes were highly up-regulated upon osteogenic differentiation and were selected for further study during hAFSCs osteogenic differentiation.

Table 1. The up-regulation of the genes of the selected ECM proteins with significant* fold changes

ECM	Fold change
Aggrecan (AGC)	6.24 ± 0.78
Decorin (DCN)	72.5 ± 40.4
Osteoblast specific factor (OSF)	35.26± 0
Thrombospondin (TSP)	13.3 ± 7.85

* All P values are less than 1×10^{-4}

2. Effects of genomically identified ECM proteins on cell differentiation. The 4 selected ECM proteins demonstrated significantly enhanced or inhibited effects on osteogenic differentiation in several ways, including concentration (Fig 2a), combination (Fig 2b), and temporal dependencies (Fig 2c).

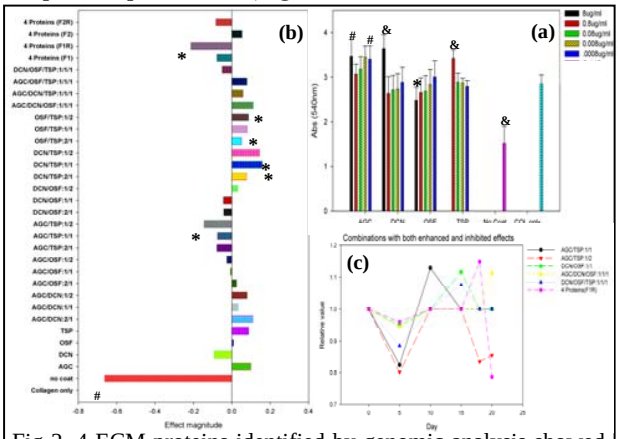

Fig 2. 4 ECM proteins identified by genomic analysis showed differential effects on HAFSC osteogenic differentiation. (a) ECM coatings with different concentrations exhibited distinct Ca productions at Day 20. (b) ECM protein combinations stimulated or slowed down the osteogenic differentiations, demonstrated by relative magnitudes over the controls at Day 20. (c) ECM coatings showed diverse temporal patterns of the effects on osteogenic differentiation over a 20-day period. (*: 0.01<P<0.05; #: 0.001<P<0.01; &: 0.0001<P<0.001)

Conclusions: ECM proteins with highly up-regulated genes identified by micro-array profiling of differentiating stem cells demonstrated certain effects on efficiency and temporal patterning of cellular differentiation. This new high throughput genomic-guided ECM molecule screening represents a new approach for ECM mimicry in tissue engineering and regenerative medicine applications.