Genomics-Guided Biomaterials Development

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Statement of Purpose:

Development of biomaterials for tissue engineering and regenerative medicine applications has focused on mimicry of the extracellular matrix (ECM) in both form, and to a much lesser extent, function. A cursory review of any major text on the ECM will demonstrate that the emphasis of much of what is known about ECM composition and function is related to only a few types of biomolecules: collagen, fibronectin, laminin, and elastin. A recent literature search of the key words, "extracellular matrix" and "biomaterial" yielded 1,432 hits. If one adds the term "biglycan," a critical ECM proteoglycan in bone formation, the number of hits drops to only 3. This begs the question, are collagen, fibronectin, laminin, and elastin the most widely studied ECM-related biomaterials because they are the most important, or because they are the most easily studied?

Most approaches to biomaterials development for tissue engineering fail to take into account the tremendous complexity of the ECM. As a result, biomaterials development has fallen behind advances in cell and molecular biology. We have recently used a line of stem cells isolated in our lab as an *in vitro* model system to study ECM formation from the perspective of the differentiating cell. By investigating gene expression of stem cells as they reach their terminal fate, 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 and tissue formation. Further, that these molecules can serve as potential targets for new biomaterials development for tissue engineering applications.

Methods:

Human amniotic fluid was collected from 14-18 week old fetuses and grown in basic media with serum. A progenitor cell was isolated and expanded. Human amniotic fluid-derived stem cells (HAFSC) were differentiated into endothelial, hepato-, myo-, and osteo-lineages using published protocols established in our labs.

Microarrays were performed on undifferentiated HAFSC lines at 20 and 30 days following myogenic and osteogenic differentiation, 14 and 30 days following hepatogenic differentiation, and 30 days following vasculogenic differentiation. RNA was isolated using RNAseB and hybridized to the Affymetrix U133A GeneChip (Affymetrix, SantaClara, CA) as described by the Affymetrix protocol.

Our analysis consisted of using a variety of computer programs as shown in Figure 1, which served to identify several ECM-related genes with significant fold changes of 20, 25, 6 for biomaterials 1, 2, 3 (Table 1), respectively, that were up-regulated upon osteogenic differentiation.

Collagen coatings containing a range of the ECM target molecules were prepared on standard cultureware by overnight incubation. HAFSC were cultured on these coatings for up to 25 days in osteogenic differentiation media (DMEM/FBS supplemented with dexamethasone, beta-glycerophosphate, and ascorbic acid-2-phosphate). At several time points, calcium production was measured by alizarin red staining.

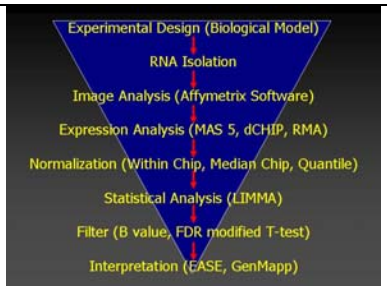

Figure 1. Methodology for microarray analysis. Data files were first analyzed with Microarray Suite 5.0 for Present/Absent detection calls. Raw data files were incorporated into dCHIP, normalized to median chip intensity, and model based expression index was computed on Perfect Match/Miss signal intensities. Probe-level data was then summarized with RMA, and differentially expressed genes were found with LIMMA. Differentially expressed genes were ranked using the B statistic and p values were adjusted using the FDR method of Benjamini and Hochberg. Genes that had a B value of greater than 1 (Log of Odds score), with a False Discovery Rate modified p value of less than 0.002 were selected as being differentially expressed.

Results and Discussion:

Microarray analysis of differentiating HAFSC provided more than 40 ECM-related genes that were universally up-regulated as these cells reached their terminal fate. HAFSC grown on coatings of these materials in a collagen carrier showed several interesting phenomenon for some of the coatings in a dose-dependent fashion. First, the onset of differentiation was faster in some of the ECM-coated wells. Second, the relative spatial orientation of cells was more homogeneous, as was matrix production (data not shown). Third, calcium production was significantly higher for two of the test materials, as is shown in Table 1.

Table 1. Calcium production of osteoblasts differentiated from HAFSC on biomaterial-coated cultureware, as measured by alizarin red staining.

	Average	p Value
Control	0.996	
Biomaterial 1	1.121	0.037
Biomaterial 2	1.159	0.007
Biomaterial 3	0.722	0.002

Conclusions:

Genomics analysis of a human stem cell model system afforded many biomaterials development targets. Evaluation of several of these compounds showed the impact of substrate-mediated differentiation. Use of genomics information to guide the development of new biomaterials for tissue engineering and regenerative medicine applications represents a new approach for ECM mimicry.