

Table 1. Proteins identified specifically in SF from OA patients that are directly involved in cartilage ECM synthesis.

Acc. No. Abbr.	Name	Cellular role
P01033 TIMP1	Metalloproteinase inhibitor 1	Prevents ECM degradation
P16112 PGCA	Aggrecan core protein	ECM component
P49747 COMP	Cartilage oligomeric matrix protein	ECM component
Q15113 PCOC1	Procollagen C-endopeptidase enhancer 1	Collagen synthesis
Q92954 PRG4	Proteoglycan-4	ECM component
Q9H9S5 FKRP	Fukutin-related protein	Glycoprotein synthesis
Q9NQ79 CRAC1	Cartilage acidic protein 1	Cartilage component

Functional Proteomics: Beads –based array system for Biomarker Discovery

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Despite the immense progress in Molecular Biology and Genetics, only a small fraction of the proteome is understood at biochemical level. Currently, a development of new methodological strategies in high-throughput format is needed for applications in Proteomics, such as Biomarker and Drug Discovery studies. These new methodologies must be able to analyze simultaneously hundreds or thousands of proteins in order to evaluate functionality, stability, interactions, relative abundance, post-transduction modifications, etc.

Our group has developed a microspheres array (Bead-based Array System, BBAS) that allow the simultaneous analysis of numerous sera and intracellular proteins. The method consist of having different populations of spheres, colored by surface-labeling with different fluorescence dyes and coupled

with different antibodies against target proteins. A wide range of available dyes could potentially be used to generate complex color codes that are analyzed by standard flow cytometres.

In the experimental process a lysate of B cells is fractioned by size exclusion chromatography (FPLC) and incubated with a 1300 populations array. Each population has a code of dyes and is specific for a particular target protein. The labeling of these target proteins with phycoerythrin (PE) allows the detection by flow cytometry.

This methodology is capable of giving information about the amount of protein present in each fraction, in addition to protein state (soluble, membrane protein, monomeric vs multimeric, phosphorylated, coupled with other proteins in functional complexes ...).