Assay for Drug Screening in Rheumatoid Arthritis

Fleming researchers have developed an actin-based cellular assay for drug discovery in treating Rheumatoid Arthritis (RA). The assay uses primary synovial fibroblasts (SF) isolated from the joints of proprietary arthritic transgenic mice overexpressing human TNF. The assay accurately quantifies the intracellular level of F-actin using fluorescently-labelled phalloidin. Reduced F-actin levels following treatment with a candidate compound indicates possible therapeutic potential for RA. The assay is fast, easy to perform with very good signal-to-noise ratio and excellent reproducibility and has the potential to develop into a high-throughput assay for screening candidate drugs in treating RA. The novelty of the assay lies in the fact that it is performed using primary arthritic cells; this offers the benefit of screening for drugs in a complex system which preserves the biological complexity of the arthritic microenvironment.

The assay has already undergone preliminary validation with commercially available RA drugs, such as methotrexate, where it has shown initial positive results.

Intellectual Property

A patent application has been filed by Fleming on deregulated genes and/or processes in inflammatory arthritis, which include genes involved in cytoskeletal reorganization. The patent provides a novel, twin high-throughput expression profiling assay in order to determine whether a test compound affects the phenotype of synovial fibroblasts taken from arthritic mouse joints in vitro.

(Priority GR 20050100526, October 19 2005)
US 12/090,999
Europe 06806493.0
Japan 2008-535987

Product Opportunity

There is the opportunity to:
- use the assay for RA drug candidate screening
- develop this assay into an off-the-shelf screening tool for therapeutic agents for RA
- develop diagnostic assay for arthritis, and other inflammatory diseases, based on the expression of genes that we have identified as being deregulated in RA

Companies that are most likely to be interested in this assay are:
- active in the following disease hubs:
  - rheumatoid arthritis
  - arthritis
  - Ankylosing Spondylitis
  - Juvenile arthritis
  - Lupus
  - Osteoarthritis
  - Autoimmune Disease
- developing inhibitors of actin, actin modulators and/or actin-related proteins that may also have applications in cancer treatment.
Technical Description

Actin cytoskeleton is critical for the maintenance of cell shape and architecture, for modulating cell motility and ultimately affects survival of the cells. Interestingly, increased F-actin polymerization has been implicated in RA pathogenesis. Fleming researchers have demonstrated that pharmacological inhibition of actin polymerization decreases potential pathogenic properties of the arthritogenic synovial fibroblast, such as proliferation, adhesion, migration and resistance to apoptosis. Thus modification of F-actin levels offers novel opportunities for therapeutic intervention in arthritis, and indeed drugs already exist that reduce F-actin as a means of treating RA (i.e. methotrexate). The novelty of this assay lies in its potential to be used as a high throughput screening tool to assess the potential therapeutic impact of drug candidates in RA in vitro.

The assay uses primary cells from the arthritic joints of the well-characterized Tg197 mouse, and by comparison with wild-type cells, measures whether F-actin levels have been reduced, thus indicating potential therapeutic potential for RA. The specific experimental set-up abolishes the need for further testing in an independent biological model, thus significantly increasing the throughput and speed with respect to currently used assays. Toxicity assays are used to eliminate the possibility of false-positive tests by confirming that reduced F-actin levels are not simply due to cell death. Once screened in-vitro, suitable drug candidates can be tested into our in vivo mouse models of RA for further verification and analysis.

Cells are isolated from mouse joints according to previously published protocols (Kontoyiannis D., Pasparakis M., Pizarro T. T., Cominelli F., Kollias G., 1999, "Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies", Immunity, 10, 387-398). They are then seeded in 96-well plates at a density of 10,000-20,000 cells/well and are subsequently treated with different pharmacological compounds for different amounts of time and using a range of drug concentrations. Following the completion of the incubation time, cells are fixed and stained with fluorescent-labelled phalloidin. Finally, the fluorescence intensity is measured by a fluorescent plate reader.

Advantages of the assay
1) Fast (only 3h to complete the assay after the treatment of cells with the compounds)
2) Reproducible
3) Sensitive
4) Easy to perform
5) Inexpensive
Preliminary Data

1) Basic principle of the assay is the quantification of the changes seen under the microscope (at identical exposure times).

2) Latrunculin A binds actin monomers near the nucleotide binding cleft with 1:1 stoichiometry and prevents them from polymerizing. Here we present results when Latrunculin A is tested using our assay:
3) Examples of compound screening using our assay

A) [Graph showing data for untreated, LatA, and various compounds under WT and Tg197 conditions after 3h treatment.]

B) [Graph showing data for untreated, LatA, and various compounds under WT and Tg197 conditions after 2h treatment.]