Attagene Profiles Chemical Effects, Toxicity With Multiplex Reporter System Assays

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NEW YORK (GenomeWeb) – More than a decade after introducing its multiplex reporter system Factorial, North Carolina-based life sciences company Attagene is continuing to demonstrate applications for the platform.

Factorial — a method described in Nature Methods in 2008 — makes it possible to accurately and reproducibly report on many proteins in parallel in a given eukaryotic cell, explained Attagene President and CEO Sergei Makarov.

The platform now serves as the basis for a range of assays that measure the activity of sets of transcription factors, nuclear receptor proteins, or G-protein coupled receptor proteins to gauge and characterize chemical effects on these cells. And together, these assays are proving useful for everything from toxicity prediction and testing to drug development, explained Makarov, who left University of North Carolina, Chapel Hill in the mid-2000s to focus on developing the Factorial system.

Since then, he and his collaborators have shown that they can generate characteristic transcription factor activity signatures that coincide with specific perturbations in the cell in response to chemical exposures, for example.

For a paper published in Sciences Advances last week, Makarov led a team of investigators from Attagene and the US Environmental Protection Agency who showed how these quantitative transcription factor activity profile (TFAP) signatures shifted in human cells in response to a wide range of compounds. They reported that similar TFAP signatures turn up for compounds prompting the same sorts of biological consequences in a cell, regardless of how much the structure of the chemical culprits resembled one another.

"If the chemicals have a common signature, this common signature signifies the effect on certain biological pathway," Makarov said, adding that "if you want to know what the biological process or pathway affected, you would just need to find a landmark compound that has the same signature and then you would put an identifier on this cluster."

Where transcriptomic experiments can be tricky to replicate from one lab or day to the next, the transcription factor activity signatures — readouts of pathway regulation — appear to be more stable and quantitative, Makarov said. The method does not rely on knowledge about the kinds of gene expression differences these transcription factor changes induce. Instead, the suite of transcription factor activity changes, analyzed alongside the large collection of TFAP signatures in Attagene's database, serve as a tipoff to the type of perturbation a compound is causing.

Attagene launched in 2001 and initially considered offering reagents or kits to its customers and collaborators. But the company quickly realized that it would need to build up sufficient stores of data to interpret the biological meaning of its Factorial platform assays.

The company began screening environmentally relevant chemicals as one of the providers for the EPA's ToxCast program more than a decade ago. In the process of participating in these types of collaborations, it has fleshed out its library of roughly 40,000 signatures by exposing a sub-clone of human liver cancer (HepG2) cells with pronounced metabolic activity to thousands of different chemicals — a collection that helps predict the consequences of new chemical exposures.

In 2004, the firm was part of a team that secured a $11.6 million grant from the National Institutes of Health to support applications of its technology to understand innate and immune response signaling components. It has since received several multimillion-dollar contracts and grants from the EPA and NIH, respectively. And in 2012, Attagene announced that it had received a $53.4 million contract to do in vivo and in vitro assays on hundreds of thousands of samples for the Environmental Protection Agency (EPA) over five years.

The EPA remains one of Attagene's primary customers, though Makarov noted that the firm has also received contracts from the US Geological Survey as well as major pharmaceutical company clients. The company's mouse model for reporting on multiple transcription factor responses in vivo was included in separate studies done in collaboration with investigators from Pfizer and from the Roswell Park Comprehensive Cancer Center.

In the new Science Advances study, the researchers described half a dozen distinct TFAP signatures including responses to mitochondrial electron transport chain inhibitors, histone deacetylase enzyme inhibitors, DNA-damaging drugs, and other compounds by several cell types. While the precise TFAP signature may differ from one cell type to the next, they found, these signatures were consist within a given cell type following treatment with compounds that interfered with the same biological processes.

"It doesn't matter how you interfere, or where you interfere, with a [given biological process], the signature is going to be the same," Makarov said. "So very different chemicals and chemical structures that produce the same biological effect would give you the same signatures."

For one of the experiments, the team compared TFAP signatures for two proposed diabetes drugs: pioglitazone, which is still on the market, and troglitazone, which been withdrawn. When treated with low concentrations of these compounds, HepG2 liver cancer cells had similar TFAP signatures. At higher concentrations of the drugs, though, the signatures diverged, and the troglitazone started producing a TFAP signature linked to mitochondrial inhibition, consistent with off-target, liver-damaging effects described for that drug.

"This is a very, very simple way to run clinical discovery … and come up with leads that are least likely to be toxic," Makarov said, noting that Attagene has generated TFAP-based identifiers beyond those presented in the paper, including a signature linked to kinase inhibitor activity.

The team plans to publish another study on the use of TFAP signatures for assessing water quality in US streams — research done as part of a contract with the EPA. Pure water should not prompt transcription factor activity shifts, Makarov explained, so transcription factor activity changes in cells can help identify suspicious water samples and provide clues to the nature of the threat.

In addition to offering TFAP as a service for investigators at regulatory agencies, pharmaceutical companies, and universities, Attagene has developed several other Factorial-based assays, including an assay designed to detect endocrine disrupting drugs based on changes in activity by dozens of human nuclear receptors, and a GPCR Factorial assay looking at G-protein coupled-receptor responses.

"We have modified the Factorial platform to produce different assays," Makarov said. "If you run chemicals through all three [transcription factor, nuclear receptor, and G-protein coupled-receptor] assays, you get an unbelievable amount of information about what these chemicals can do … and how dangerous it can be, in the case of environmental chemicals."