Innovations

Cell biology goes commercial

Cytokinetics, Inc.

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Every company wants a drug like Taxol. Bristol-Myers Squibb sold $329 million of the anticancer agent in the first quarter of 1999 alone. Now Cytokinetics, Inc. (South San Francisco, California) is seeking a new, improved Taxol. Their approach is unique. Instead of aiming at microtubules, the cellular train tracks attacked by Taxol and a slew of similar anticancer compounds, Cytokinetics is targeting all the other proteins of the cell's cytoskeleton. The company claims that these new targets will be more specific to the disease than the ubiquitous microtubules, thus reducing toxicity.

The three filament systems of the cytoskeleton — actin, microtubules, and intermediate filaments — criss-cross the cell and give the cell its shape and mechanical strength. But the cytoskeleton is no longer seen simply as a rusty neglected scaffolding. Dynamic filaments are integral to cell division, movement and signaling in normal and diseased cells. "Basically the cytoskeleton is involved in most of the important things that the cell does," says Larry Goldstein, a biologist at the University of California, San Diego, and one of the founders of Cytokinetics. An explosion in knowledge about the cytoskeleton — the identification of new components and determination of their function — has given Cytokinetics the raw materials it needs. "Right now there's a richness of understanding of the role of the cytoskeleton," says Cytokinetics co-founder and CEO James Sabry. "That for us represented a real opportunity."

Friendly beginnings

Sabry is a young but confident newcomer to the world of business. He started his scientific career with a brief stay at Roche in Switzerland before heading to graduate school and medical school at the University of California at San Francisco, where he met Ron Vale, another Cytokinetics co-founder. Next came a postdoctoral fellowship with the final Cytokinetics co-founder: Jim Spudich of Stanford University (Palo Alto, California).

After three years of business training, Sabry was eager to commercialize some science. "I'm fascinated by how biology and medicine intersect, and I've always had an inner entrepreneur," he says. "The opportunity to build a new company in an area that I think has great opportunity was too much to pass up."

The cytoskeleton has proven to be a profitable drug target. But can it be more intelligently exploited?

The scientific co-founders were happy to put someone else in charge. "The investors asked 'which one of you is going to pull a McKnight?' but none of us were wanting to leave academia," says Goldstein. "James is very entrepreneurial, and he has the academic and medical background. Plus the four of us have worked together. That makes it fun as well as productive."

Let the screens begin

The company structure is a combination of drug discovery and informatics, with the informatics providing some short-term income. The company started operations in July 1998, and is already doing high-throughput screening against purified proteins, including seven anticancer targets, six cardiovascular targets and one fungal target. Cytokinetics has small molecule hits for all the targets, and some of the anticancer hits work against cells *in vitro*. Sabry is not disclosing the details of the screening method, but he says that it can assay up to 120,000 compounds in one day.

The first major focus is cancer and, more specifically, kinesin proteins. Kinesins are motors that run along microtubules. They deliver cargo, build the mitotic spindle, and distribute chromosomes into two daughter cells. The kinesins have a well-defined activity that can be assayed, and some of them are mitosis specific, unlike microtubules.

The possibilities do not stop with kinesin. Over 5000 cytoskeletal proteins may exist in man, and inhibitory drugs exist only for tubulin, the building block of microtubules. "The anti-microtubule drugs are a proof of principle," says Spudich, "but [the microtubule] is probably the worst of the 5000 things you'd want to target."

Old drugs and new targets

Still, Taxol and taxotere (a chemical relative of Taxol made by Rhône-Poulenc Rorer (RPR)) are both blockbuster drugs, and close behind them are developmental anti-microtubule compounds such as the epothilones, cryptophycins, dolastatins, and discodermolide. "Anti-microtubule drugs are still seen as a deep well where we are still getting good new compounds," says Dan Sackett of the National Institutes of Health (NIH; Bethesda, Maryland).

"There's a tremendous amount of activity in this area," says Susan Horwitz (Albert Einstein College of Medicine, Bronx, New York). In 1979, Horwitz determined the mechanism of action of Taxol. Now she says researchers are hoping to make smaller compounds that can work in oral formulations and against different types of tumors. "All these things," she says, "are in the making."

Cecile Combeau, a senior scientist at RPR, says that RPR is trying to extend the indications for taxotere and to find compounds that are not disposed of by resistance proteins. "Then I think that's the end of

tubulin as a target," she says. "We are more interested in other approaches — apoptosis, angiogenesis, signal transduction and mitotic kinases."

Combeau says that RPR has not done targeted assays against individual cytoskeletal proteins other than tubulin. "People here think that targeting these proteins would lead to the same toxic effects seen with anti-microtubule agents," she says. Combeau believes that finding kinesin inhibitors will be easy. The harder task will be finding inhibitors that are specific to mitotic kinesins, so kinesins that drive secretion and transport in nondividing cells are not affected. Sabry says, "we've already done that, and [the compounds] are remarkably specific."

The company's first kinesin inhibitor was adociasulfate-2 (AS-2), which Goldstein isolated in 1988 from a marine sponge. AS-2 inhibits the kinesin–microtubule interaction rather than the kinesin active site. It is not a drug candidate, as it inhibits a number of different kinesins equally, and is cell impermeable.

A mitosis-specific drug would eliminate one significant side effect of anti-microtubule drugs: the neurotoxicity that is dose limiting for the vinca alkaloids. But for taxotere the dose-limiting side effect is a reduction in neutrophil levels presumably caused by mitosis inhibition. A kinesin drug may run into similar problems, and NIH's Sackett says that in the meantime the problem may be bypassed by a tubulin drug with improved tissue distribution properties. All anticancer drugs, he notes, are at the mercy of the poorly understood issue of tissue distribution.

The biggest question of all is whether any kinesin inhibitor will work as an anticancer drug. "They are really taking a chemical genetic approach in that they are taking a nonvalidated target, screening for inhibitors, and then seeing if inhibitors do anything," says Randy King (Harvard Medical School, Boston, Massachusetts), who is screening in frog extracts for anti-mitotic compounds that do not affect microtubules. Goldstein did show that AS-2 inhibits mitosis in fruit flies and, although it is a long way from flies to cancer, Sabry says, "we're way ahead of fruit flies now."

Beyond kinesin

The founders of Cytokinetics do not see the cytoskeleton as limiting their vision. "I believe the cytoskeleton will touch on almost all diseases at some level," says Spudich. "Nothing is really ruled out in my mind."

Targets other than kinesin are being selected based on existing cell biological knowledge, and Sabry claims the company has targets that are both mitosis specific and cancer specific. For some targets Cytokinetics has been the first to clone the human version of the gene, and so can obtain a composition of matter patent. Spudich says the company will be watching the information emerging from the human genome project for further target ideas.

In its cardiovascular program, Cytokinetics is hoping to find an activator of muscle contraction to aid in congestive heart failure. Although activators are notoriously hard to find, an inhibitor of an inhibitory protein may work just as well. The focus in the antifungal project will be on cytoskeletal elements that differ between the host and fungal cells, with a special emphasis on organelle transport and filamentous growth. The latter is essential for the virulence of fungi such as *Candida*, and is also an interest of a start-up called Microbia, Inc. (Cambridge, Massachusetts).

An informatics back-up

Cytokinetics is developing a cell-based screening and database system called Cytometrix to prioritize its own compounds and to spread the company's financial risk. Cytometrix detects changes in the cell cycle, protein trafficking, apoptosis, adhesion and endocytosis in response to drug treatment, at a rate of 1500 samples per day. The speed is projected to increase tenfold by the end of 1999. "There has to be something between getting hits and [testing in] animals," says Sabry. "Cytometrix is a post high-throughput screening filter, before you go to animals."

Cytometrix will be offered as a contract service, starting in 2000 or 2001. The company is building a database of cell responses to chemicals with known mechanisms of action, and Sabry says the database should be a good predictor of the mechanism of action of novel compounds. Similar concepts are used in transcriptional profiling using DNA chips. "We see these approaches as complementary," says Sabry. "If I were a Merck or Pfizer I would want both."

The chips are best at testing the response of thousands of genes to one or a few conditions. There are fewer read-outs from Cytometrix, but it can test thousands of samples. Furthermore, says Sabry, "the transcription read-out is a surrogate for the biology, whereas we are measuring the biology directly."

Competition may be heavy in phenotype testing. Cellomics, Inc. (Pittsburgh, Pennsylvania) has a similar high-throughput system (see *Chem. Biol.* **5**, R205). Michael Sheetz (Duke University, Durham, North Carolina) likes the database idea, but says that "the question is if everyone and their brother is going to try that approach."

The founders of Cytokinetics may have chosen two areas likely to attract many other companies, but they remain confident of their ability to stay ahead. "The amazing thing is that there weren't already 30 companies doing this," says Spudich. "I think there will be plenty of people entering this area, but quite frankly I think there is plenty of room."

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