β testing adenosine receptor agonists

By Tim Fulmer, Senior Writer

Researchers at the **University of California, San Francisco** have used a zebrafish screen to identify an adenosine receptor agonist that improved both β cell regeneration and glucose control in diabetic mice.[1] The discovery could open up a new indication for a class of compounds that until now have been developed mainly for inflammatory and cardiovascular disorders.

Despite efforts to discover small molecules that trigger regeneration of insulin-secreting β cells, the compounds have eluded diabetes drug developers because *in vitro* screens generally cannot reflect the complex, interrelated pathways and multiple types of progenitor cells that take part in β cell development.

Thus, a UCSF team led by Didier Stainier and Olov Andersson turned to a phenotype-based *in vivo* screen. "It allows for the discovery of compounds that target various cellular mechanisms of regeneration, which is important because regeneration of β cells can potentially occur through several mechanisms," Andersson told *SciBX*.

Stainier is professor of biochemistry and biophysics at UCSF. Andersson has been a postdoctoral research fellow in Stainier's lab since 2008 and is transitioning to an assistant professor post at the **Karolinska Institute**.

In prior work, Stainier and colleagues had designed a zebrafish system that allowed for selective ablation of cell types to study their effects on zebrafish development and regeneration.[2] They adapted that system to selectively ablate β cells.

In zebrafish embryos with ablated β cells, the researchers screened about 7,000 small molecules for their ability to double the number of β cells after only 2 days of regeneration. Five compounds reached that threshold, of which four targeted adenosine signaling and adenosine metabolism.

The most potent was 5′-N-ethylcarboxamidoadenosine (NECA), which significantly increased β cell regeneration compared with vehicle control (p=0.0002). In the same zebrafish embryos, NECA restored normal glucose levels significantly faster than vehicle at both two and three days post-treatment (p=0.0031 and p=0.0292, respectively).

Additional mechanistic studies showed that the effects of NECA were mediated by the adenosine A2aa receptor, a zebrafish homolog of the adenosine A_{2A} receptor ($ADORA_{2A}$) in humans.

NECA had similar effects in mammals. In mouse islet cells, the molecule significantly increased β cell proliferation compared with vehicle (p<0.01). In a mouse model of chemically-induced diabetes,

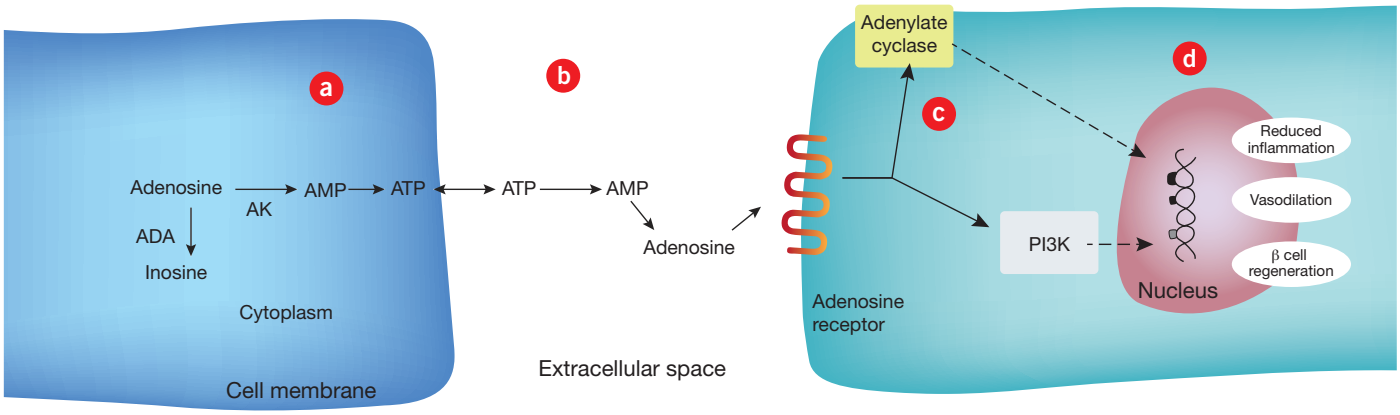

Figure 1. Agonizing β cell regeneration. A paper published by **University of California, San Francisco** researchers in *Cell Metabolism* suggests agonists of the adenosine A_{2A} receptor ($ADORA_{2A}$) can trigger regeneration of pancreatic β cells and thus could help treat diabetes. $ADORA_{2A}$ plays a central role in adenosine metabolism and signaling.

Breakdown of adenosine by adenosine kinase (AK) and adenosine deaminase (ADA) generates essential metabolic molecules, including inosine, AMP and ATP [**a**]. When ATP is released into the extracellular space, it can be converted back to adenosine, which binds and activates $ADORA_{2A}$ on the surface of neighboring cells [**b**]. $ADORA_{2A}$ then triggers downstream signaling via G proteins and kinases, such as adenylate cyclase and phosphoinositide 3-kinase (PI3K) [**c**], which finally leads to expression of multiple genes involved in β cell regeneration, inflammation and vasodilation [**d**].

The UCSF group used a zebrafish screen to identify small molecules that acted on the adenosine pathway. The most potent compound, 5′-N-ethylcarboxamidoadenosine (NECA), agonized $ADORA_{2A}$ and increased β cell proliferation in zebrafish and diabetic mice.

NECA also improved both β cell proliferation (p=0.0019) and glucose control (p<0.001).

The findings were published in *Cell Metabolism*.

Getting more selective

Despite the strong mouse data, NECA is ill suited as a drug candidate because it nonselectively agonizes all adenosine receptors, not just $ADORA_{2A}$.

"A selective A_{2A} agonist should thus be evaluated," said Joel Linden, professor of inflammation biology at the **La Jolla Institute for Allergy & Immunology**. Linden and colleagues have shown that selectively agonizing $ADORA_{2A}$ has anti-inflammatory effects in several mouse and cell culture models, including pulmonary inflammation,[3] graft-versus-host disease (GvHD)[4] and bacterial gastritis.[5]

Linden also has shown that agonizing $ADORA_{2B}$ contributes to insulin resistance in mice.[6]

Andersson said he is studying adenosine agonists that are more specific than NECA in the zebrafish. Those include "A_{2A} receptor agonists that have been in clinical trials for other indications and have already gone through safety assessments," he said. He declined to provide details about the compounds.

At least three $ADORA_{2A}$ agonists have progressed into—or through—the clinic as vasodilators used as adjuncts to myocardial perfusion imaging (MPI) studies. These include Lexiscan regadenoson, which is marketed by **Gilead Sciences Inc.** and **Astellas Pharma Inc.**; **Pfizer Inc.**'s binodenoson, which is in registration; and **Forest Laboratories Inc.**'s apadenoson, which is in Phase III testing.

Given the broad effects even selective $ADORA_{2A}$ agonists can have on cell types including immune and cardiac cells, it might be advisable to design $ADORA_{2A}$ agonists that target the receptor specifically on β cells, according to Cord Dohrmann, CSO of **Evotec AG**.

Dohrmann declined to say how that might be accomplished. Published approaches to selectively target small molecules to β cells in animals have generally relied on peptides and peptide fragments.[7,8]

In 2011, Evotec announced a deal with **Harvard University** and the **Howard Hughes Medical Institute** to discover and develop diabetes therapies targeting pancreatic β cell regeneration and replication. Earlier this year, Douglas Melton and colleagues published some of the first results of that collaboration in the *Proceedings of the National Academy of Sciences*.

Melton, a professor of molecular and cellular biology at Harvard and co-director of the **Harvard Stem Cell Institute**, used a screening platform based on freshly isolated rat islet cells to screen for small molecules that promoted replication of β cells. They identified a class of adenosine kinase inhibitors that promoted replication of cultured primary β cells from mice, rats and pigs.[9]

Adenosine kinase functions upstream of $ADORA_{2A}$ in the adenosine signaling pathway (*see* **Figure 1, "Agonizing β cell regeneration"**). The adenosine kinase inhibitors are licensed to Evotec, Melton told *SciBX*. Dohrmann declined to disclose the company's plans for the inhibitors.

Getting more human

Moving forward, the researchers will need to understand how the adenosine pathway works in human islets and whether long-term $ADORA_{2A}$ agonism is safe in mammals.

Determining relevance of the adenosine signaling pathway to human β cell proliferation is a critical next question, Andrew Stewart told *SciBX*. Thus, he suggested testing $ADORA_{2A}$ agonists in human fetal or neonatal cadaveric islets or, alternatively, in β cells derived from human induced pluripotent stem (iPS) cells or embryonic stem cells.

Stewart is professor of medicine at the **University of Pittsburgh School of Medicine** and chief of the Division of Endocrinology and Metabolism.

Stewart and colleagues at the **Broad Institute of MIT and Harvard** have developed a high throughput platform based on cultured human islets that they are using to screen for small molecule inducers of β cell proliferation.[10] Stewart said a primary focus of his own work is developing ways to activate cell cycle proteins like cyclin dependent kinase 6 (CDK6), which has been shown to induce β cell proliferation in cultured human islets.[11,12]

"From a safety standpoint, it will be critical to understand the effects of chronic stimulation of the A_{2A} receptor in rodents and monkeys."

—Hui Tian, NGM Biopharmaceuticals Inc.

"From a safety standpoint, it will be critical to understand the effects of chronic stimulation of the A_{2A} receptor in rodents and monkeys," said Hui Tian, VP and head of research at **NGM Biopharmaceuticals Inc.**

Earlier this year, NGM and **Daiichi Sankyo Co. Ltd.** partnered to discover and develop therapeutics that modulate β cell regeneration to treat diabetes. NGM is using its large-scale, high throughput, *in vivo* screening platform to discover and evaluate therapeutic candidates in rodents, said Tian.

"We are primarily focused on biologics—peptides, proteins and antibodies—since we believe they can achieve better target or cell specificity than small molecules," he added.

Finally, Linden pointed out that islet regeneration by itself "does not solve the problem of autoimmunity and immune rejection of new islets."

Andersson acknowledged that "there might be a need to combine a β cell–regenerative therapy with an immune suppressant" in type 1 diabetes, adding that "a β cell–regenerative therapy might also be advantageous for patients with late-stage type 2 diabetes who have reduced β cell mass and where immune responses are less prominent."

The findings in the *Cell Metabolism* paper are not patented, Andersson said.

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